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DISEASE-DRUG INTERACTIONS: PHARMCOKINETICS AND PHARMACODYNAMICS OF SOTALOL AND LIDOCAINE IN THE PRESENCE OF INFLAMMATORY CONDITIONS

BY

KENNETH M. KULMATYCKI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMET OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR IN PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES

Faculty of Pharmacy and Pharmaceutical Sciences

Edmonton, Alberta, Canada

Fall, 2000

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TITLE OF THESIS:	**Disease-Drug Interactions: Pharmacokinetics and Pharmacodynamics of Sotalol and Lidocaine in the Presence of Inflammatory Conditions**
DEGREE:	**Doctor of Philosophy**
YEAR THIS DEGREE GRANTED:	**Fall 2000**

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"I hope my achievements in life shall be these -- that I will have fought for what was right and fair, that I will have risked for that which mattered, and that I will have given help to those who were in need that I will have left the earth a better place for what I've done and who I've been."

C. Hoppe

ABSTRACT

Inflammation increases concentrations of α_1-acid glycoproteins and decreases hepatic clearance, both of which can result in greater drug concentrations. Preliminary data from our laboratory, however, suggests that despite higher concentration, a reduced potency of verapamil and propranolol may result as a response to inflammatory conditions. Both drugs are highly bound to plasma proteins thus reduced response may be due to an inflammation-induced reduction in free fraction or receptor activity. In addition, virtually nothing is known about responsiveness of cardiac potassium channels in inflammation. Thus, we chose sotalol, β-adrenergic/cardiac potassium channel blocker, negligibly protein bound and eliminated via the renal route, to determine whether altered response to drug is pharmacokinetic dependent or effect of inflammation on potassium channel activity. Similar to propranolol and verapamil, lidocaine, cardiac sodium channel blocker, undergoes extensive first-pass metabolism, is highly protein bound, however, unlike the former two drugs, is metabolized mainly by cytochrome P450 (CYP) 2C subfamily in the rat. Therefore, to determine whether inflammation-induced alterations may be universal, lidocaine pharmacokinetics and cardiodynamics were investigated in rats with inflammatory disease.

Pharmacokinetic-pharmacodynamic (electrocardiogram, ECG) studies were then performed by administering sotalol to healthy and chronic arthritic (*Mycobacterium butyricum*-induced) adult male Sprague-Dawley rats. Sotalol racemate, R (β-adrenergic/potassium channel blocker) and S (potassium channel blocker) sotalol were administered to healthy and acutely inflamed (interferon$_{\alpha 2a}$-treated) rats. Lidocaine and verapamil (positive control) and placebo were administered to healthy and acutely

inflamed (interferon$_{\alpha 2a}$-treated) rats. Serial blood samples were collected via the jugular vein. Lead I ECG was used to record the PR, RR (heart rate) and QT intervals. Both chronic arthritis and interferon$_{\alpha 2a}$-induced inflammation decreased effect of racemate and R-sotalol on PR and QT intervals. Responsiveness of QT intervals to S-sotalol was decreased in rat with acute inflammation confirming reduced potassium channel sensitivity. No significant differences in sotalol enantiomer pharmacokinetics were observed between control and inflamed rats. Thus, altered ECG responses to sotalol despite unaltered pharmacokinetics may be attributed to the influence of pro-inflammatory cytokines on the action of drug on both β-adrenergic and potassium channel receptors. Unlike verapamil, disposition and responsiveness to lidocaine was not affected by the cytokine-induced inflammation. This indicates a selective effect of interferon$_{\alpha 2a}$-inflammation on CYP isozymes and cardiovascular receptors suggesting the influence of inflammation is not universal.

Dedication

To my mother and father for their guidance and wisdom.

ACKNOWLEDGEMENTS

I would like to thank Dr. Fakhreddin Jamali for his expert advice and patient guidance throughout my graduate studies. His support and understanding are deeply appreciated. I found Dr. Jamali's dedication to science and enthusiasm for discovery truly inspirational. I consider an honor to have studied under his supervision.

I would also like to thank my fellow colleagues: Pat Mayo, Micheal Guirguis, Sharon Mitchell, Dr. Jaber Emamibafrani, Kassim AbouChehade, Noriko Daneshtalab, Yaman Dakhel and Spencer Ling. I would especially like to thank Dr. Ray Lovlin our previous laboratory manager for his support.

I also thank my family for their words of encouragement, guidance, and kindness.

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LIST OF ABREVIATIONS AND SYMBOLS

AI	Arthritic Index
α	Alpha
AAG	α_1-acid glycoprotein
AUC	Area under the plasma concentration-time curve
AUC_{0-6}	Area under the plasma concentration-time curve from 0 to 6 h
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from 0 hours to infinity
AUEC	Area under the effect-concentration time curve
AV	Atrioventricular
β	Terminal elimination rate constant
°C	Degrees Celsius
C18	18 consecutive carbon-carbon bonds
C	Substrate concentration
CAST	Cardiac Arrhythmia Suppression Trial
CL/F	Body clearance after oral doses
CL_{oral}	Oral clearance
cm	Centimeters
C_{max}	Maximum plasma concentration after oral doses
CRP	C-reactive protein
CYP	Cytochrome P450

ECG	Lead I Electrocardiogram
E_{max}	Maximum Effect
ESR	Erythrocyte sedimentation rate
F	Absolute bioavailability
g	Centrifugal force X *g*
g	grams
h	hour(s)
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
i.d.	Internal diameter
IL	Interleukin
IL-1ra	Interleukin 1 receptor antagonist
in	Inch
IFN	Interferon
iu	units
KCl	Potassium Chloride
kg	Kilogram(s)
L	Litres
LPS	Lipopolysaccaride
MEGX	Monoethylglycinexylidide
M	Molar
µg	Micrograms
µl	Microliters

μm	Micrometer
μM	Micromolar
mg	Milligrams
min	Minutes
ml	Milliliters
mm	Millimeters
mM	Millimolar
msec	Millisecond
$NADP^+$	β-Nicotinamide adenine dinucleotide phosphate, oxidized form
$NADPH_2$	Nicotinamide adenine dinucleotide phosphate, reduced form
NaOH	Sodium hydroxide
NEIC	S-(+)-1-(1-napthyl)ethyl isocyanate
nm	Nanometers
NSAIDs	Nonsteroidal antiinflammatory drugs
o.d.	Outside diameter
ODS	Octadecylsilane
OH	Hydroxy
%	Percent
p	Probability value or the degree of rarity of a test result given that the null hypothesis is true
P-gp	P-glycoproteins
pH	Negative base 10 logarithm of the hydrogen ion concentration
pKa	Negative logarithm of the acidic dissociation constant

r^2	Pearson correlation coefficient squared
SA	Sinoatrial
SWORD	Survival with oral d-sotalol
t_{max}	Time to maximum plasma concentration
TNF	Tissue necrosis factor
$t_{1/2}$	Elimination half-life
µl	Microliter
UV	Ultraviolet
Vd	Volume of distribution
Vd/F	Volume of distribution after oral doses
v/v	Volume per volume

Chapter 1

INTRODUCTION

Pharmacokinetics describes the time course of a drug in the body resulting from the dose administered. Pharmacodynamics describes the intensity of drug response in relation to concentration (Derendorf and Meibohm, 1999). The relationship between plasma concentration of drug and intensity of pharmacological effect forms the basis of rationale drug therapy (Gibaldi *et al.*, 1971). It is generally thought that a direct or indirect relationship exists between drug concentration and response. Thus, pharmacokinetics is accepted as a surrogate marker of pharmacodynamics hence higher drug concentrations are considered to cause greater effect or toxicity. However, drug concentrations have been shown to vary with disease (Schneider *et al.*, 1979; Levy, 1998) suggesting that pharmacokinetic observation may not necessarily reflect drug activities. Thus, employing pharmacokinetics to predict drug response may not always be appropriate. To have a better understanding of the concentration-effect relationship, pharmacokinetic-pharmacodynamic studies usually begin in phase I clinical trials (Lesko and Williams, 1994). Despite the integration of pharmacokinetics and pharmacodynamics this early in the drug development process, surprisingly, these studies are usually conducted with either healthy volunteers or patients who only have the disease of interest (Lesko and Williams, 1994). Therefore, the influences of multiple diseases on drug disposition and activities may not be known predisposing patients to experiencing inappropriate pharmacotherapy. This is especially relevant to the elderly population who despite having a greater incidence of multiple drug therapy are usually excluded from clinical drug trials (Schmucker and Vesell, 1999).

Cardiovascular diseases accounted for 42% of all deaths annually in 1993 (Alexander *et al.*, 1998). These diseases resulted in $126 billion dollars in health care

expenditures in the United States (Alexander *et al.*, 1998). One in every three men and one in every ten women can expect to develop some type of cardiovascular disease before reaching age 60 (Alexander *et al.*, 1998). It is estimated that approximately 690 million people worldwide suffer from hypertension (Mulrow, 1999). Rheumatic diseases are estimated to affect 11 % of the world population (Kelley *et al.*, 1997). With this in mind, it is not surprising that in North America, over 20 million people are treated for concurrent hypertensive/inflammatory disease (MacFarlane *et al.*, 1995). Therefore, inflammatory diseases can have a significant impact on cardiovascular drug therapy, hence, patient outcomes.

Inflammatory conditions and inflammation-induced pathophysiological changes have been shown to inhibit clearance of various cardiovascular drugs in both experimental animals and humans (Piquette-Miller and Jamali, 1995; Schneider *et al.*, 1981). Inflammation causes increased concentrations of α_1-acid glycoproteins (AAG) and reduced intrinsic hepatic clearance, (Belpaire *et al.*, 1989) both of which can result in increased area under the curve (AUC). This reduced clearance of drug in laboratory animals and patients with inflammation signifies greater effect or toxicity. Preliminary data from our laboratory, however, suggests that despite increased concentrations, a reduced potency of calcium channel (Mayo *et al.*, 1996) and β-adrenergic (Guirguis and Jamali, 1996) antagonists may result, as a response to inflammatory conditions. This may be due to mechanisms such as reduced free fraction and decreased receptor responsiveness. Both propranolol and verapamil are highly protein bound and since inflammation increases AAG and subsequently increases protein binding (Piafsky *et al.*, 1978). Therefore, the reduced responsiveness of β-adrenergic receptors and calcium

channels in inflammation may be due to decreased free drug interacting with the β-adrenergic and cardiac calcium channel receptors (Belpaire *et al.*, 1989). However, *in vitro* evidence indicates a decreased β-adrenergic and cardiac calcium channel responsiveness in the presence of pro-inflammatory cytokines (Lui *et al.*, 1999; Lui and Schreur, 1995; Krown *et al.*, 1995). Thus, alteration of drug response in laboratory animals with inflammatory disease (Mayo *et al.*, 1996; Guirguis and Jamali, 1996) may perhaps be due to alterations in protein binding or cardiovascular receptor function. In addition, it is not known whether this decreased response to cardiovascular drug is universal or specific to those thus far reported.

The purpose of this thesis is to determine whether reduced response to drug observed in laboratory animals is due to pharmacokinetic or pharmacodynamic changes and whether inflammation alters cardiac potassium channel response to drug. Also, whether the reduced clearance and responsiveness to propranolol and verapamil in laboratory animals with inflammatory diseases are observed with other highly protein bound and extensively metabolized cardiovascular drugs. To accomplish these objectives pharmacokinetics and pharmacodynamics of sotalol and lidocaine were determined in healthy and inflamed rats.

Unlike propranolol and verapamil, sotalol, a racemic nonselective β-adrenergic/cardiac potassium channel blocker is negligibly bound to plasma proteins and eliminated mainly unchanged via the renal route (Anderson and Prystowsky, 1999). Thus, inflammation is not expected to reduce its clearance. Hence, pharmacodynamic assessment can be carried out in the absence of complications of altered pharmacokinetics. In addition, since sotalol is both a β-adrenergic and cardiac potassium

channel blocker, a study of its actions on cardiac indices during inflammation may reveal whether, similar to β-adrenergic and calcium channel antagonists, potassium channels are altered by inflammatory conditions. Interestingly, both R-sotalol and racemate have nonselective β-adrenergic and cardiac potassium channel blocking activities but only S-sotalol is a pure potassium channel blocker. Hence, taking advantage of stereochemistry, the effect of inflammation on cardiac potassium channels may be confirmed by administering individual enantiomers.

Lidocaine, propranolol and verapamil are candidates for inflammation-induced pharmacokinetic changes since these drugs are highly bound to plasma proteins and undergo extensive presystemic hepatic metabolism. However, unlike propranolol and verapamil, lidocaine is metabolized mainly by cytochrome P450 (CYP) 2C subfamily in the rat and blocks fast sodium channels in Purkinjie fibers and ventricular muscle (Smith, 1991; McEvoy, 1999). Thus, effect of inflammation on pharmacokinetics and cardiodynamics of lidocaine was investigated to determine whether acute inflammation influences drugs metabolized in the rat by CYP2C subfamily and whether cardiac sodium channel responsiveness is decreased in acute inflammatory conditions. Rat models of inflammation used were *Mycobacterium butyricum* induced chronic arthritis and acute inflammation caused by inoculation with interferon $(IFN)_{\alpha 2a}$. Before discussing the rationale and objectives of this thesis, previous research will be discussed.

1.1 Background

1.1.1 Inflammation

Inflammation is a physiological response to various stimuli such as infection or trauma (Kuby, 1997). Inflammation may be acute or chronic depending on the nature of this stimulus. The inflammatory response is characterized by increased capillary blood flow and permeability allowing various cells and fluid to leave the capillaries and enter the affected region resulting in swelling, redness, heat and pain (Davies *et al.*, 1999). The increased capillary blood flow and permeability enables cellular and humoral components of the immune system to enter the affected area, assist in removal of bacteria, and repair connective tissues (Kelley *et al.*, 1997).

Cytokines are hormone-like proteins that are involved in regulating immune and inflammatory responses. Secretion of cytokines by T-lymphocytes plays an important role in the pathogenesis of inflammation. A disruption of the $CD4^+$ T-lymphocyte balance [i.e., T helper (Th) 1 and T helper (Th) 2 cells] occurs in inflammatory disease. Th1 cells produce interferon (IFN)γ, interleukin (IL)-2, tumor necrosis factor (TNF)β or lymphotoxin, induce cell-mediated immunity and suppress the differentiation of Th2 cells (Morel and Oriss, 1998; Miossec *et al.*, 1996). Increased secretion of IFNγ by Th1 cells stimulates neutrophils to release pro-inflammatory cytokines [e.g., TNFα and IL-1β] which have significant roles in triggering and maintaining the inflammatory response (Cassatella, 1995). Interestingly, neutrophils are unable to produce two of the most potent anti-inflammatory cytokines, IL-10 and IL-13, which may account for the persistent inflammatory response observed with increased neutrophil expression (Reglier *et al.*,

1998). Th2 cells produce IL-4, IL-5, IL-6, IL-10, IL-13 induce humoral and parasitic immunity and suppress the differentiation of Th1 cells (Morel and Oriss, 1998).

Various diseases have been attributed to a T helper cell imbalance. For example, rheumatoid arthritis, multiple sclerosis, thyroiditis and insulin-dependent diabetes mellitus are associated with increased Th1 cell activity. Interestingly, allergy, asthma, scleroderma, and systemic lupus erythematosus are associated with increased Th2 cell activity (Miossec *et al.*, 1996; Munoz-Fernandez and Fresno, 1998; Kuby, 1997) thus both Th1 and Th2 cells can be involved in disease. Induction of Th1 cells results in release of pro-inflammatory cytokines that, in turn, induce cytotoxic and inflammatory reactions. Disruption of the T-lymphocyte balance resulting in increased activity of Th1 cells is caused by either direct stimulation, an antigen, or via IL-12 secreted by macrophages resulting in the release of inflammatory mediators. The Th1 cells then by direct contact or via secretion of IL-2, TNFβ, and IFNγ stimulate monocytes to secrete IL-11 and IL-6. Interleukin-6 then stimulates hepatocytes to increase production of acute-phase proteins such as C-reactive proteins (CRP), serum amyloid A, fibrinogen, AAG and decrease synthesis of other proteins such as albumin. The activated monocytes stimulated by Th1 cells may also secrete IL-12. That in turn, stimulates natural killer lymphocytes to secrete IFNγ which activates macrophages and inhibits Th2 cell proliferation (Morel and Oriss, 1998; Kelley *et al.*, 1997). In addition, monocytes activated by Th1 cells secrete several types of growth factors, IL-1, TNFα, chemokines and matrix metalloproteases that may contribute to the inflammatory response and tissue damage. In contrast to Th1 cells, IL-4, IL-10, and IL-13 secreted by Th2 cells stimulates monocytes to produce IL-1 receptor antagonist (IL-1ra), IL-1 soluble receptor (IL-1sr),

TNF soluble receptors and tissue inhibitor of matrix metalloproteases (Kelley *et al.*, 1997; Firestein, 1999). Thus, Th2 cells in some instances may function as anti-inflammatory cells. Mediators secreted by monocytes may act on synoviocytes and chondrocytes to produce substances such as proteases, arachidonic acid metabolites, collagen, proteoglycans and on endothelial cells to initiate cell adhesion of leukocytes and induce angiogenesis (Kelley *et al.*, 1997).

Inflammatory diseases and pro-inflammatory cytokines can influence P-glycoproteins (P-gp) which are membrane-bound ATP-dependent transporters that function as efflux pumps preventing substances from entering cells. (Ambudkar *et al.*, 1999). A decreased hepatic expression and activity of P-gp is reported to occur in livers from rats with lipopolysaccaride (LPS) and turpentine-induced inflammation (Piquette-Miller *et al.*, 1998). The reduced P-gp expression and activity was attributed to over expression of pro-inflammatory cytokines in the inflamed rat. In contrast, rat hepatocytes were cultured in the presence and absence of TNFα (Hirsch-Ernst *et al.*, 1998). Exposure of hepatocytes to the cytokine resulted in greater P-gp isoform mdr (multidrug resistance) 1b expression. It was concluded that stimuli causing elevated TNFα concentrations enhances the capacity of the liver for transport of exogenous and endogenous mdr1 substrates. Thus, effect of inflammatory disease and inflammation mediators may vary with the type of study conducted and as shown with colon carcinoma the cell line investigated. Pro-inflammatory cytokines TNFα, IL-2 and IFNγ are reported to reverse multidrug resistance in human colon carcinoma cells (Stein *et al.*, 1996). The authors suggested that modulation of multidrug resistance by TNFα, perhaps, might increase effectiveness of drug treatment for colon carcinoma by preventing efflux of drug from the

cancer cell. P-gp has also been observed to participate in the transport of cytokines in peripheral T-lymphocytes (Drach *et al.*, 1996). An increased expression and activity of P-gp on T-lymphocytes in elderly compared to younger individuals is also reported (Aggarwal *et al.*, 1997). It was concluded that the greater expression and function of these transporters may play a role in increased cytokine concentrations observed in aging since P-gp secrete pro-inflammatory cytokines from T-lymphocytes. Interestingly, increased expression of P-gp on T-lymphocytes has also been reported to correlate with drug resistance in rheumatoid arthritic patients (Yudoh *et al.*, 1999). Therefore, inflammatory conditions and pro-inflammatory cytokines may have a significant influence on P-gp expression and function that may affect drug disposition and activity.

Pro-inflammatory cytokines such as IFNγ, TNFα and IL-1β induce formation of nitric oxide, a free radical that is involved in the cardiopulmonary, nervous and immune systems (Cochran *et al.*, 1996). Nitric oxide is toxic to bacteria, therefore, is an important factor in host defense (Kuby, 1997). However, increased inflammatory cytokine expression and nitric oxide concentrations in the pro-inflammatory events during septic shock can result in multiple organ system failure (van der Poll and van Deventer, 1999; Montegut *et al.*, 1995). In addition, depression of myocardial function by IL-1β plus TNFα is reported to be mediated by excess nitric oxide production (Schultz *et al.*, 1995). Nitric oxide and its breakdown products i.e., peroxynitrite oxidize heme groups on CYP isozymes preventing metabolism of various drugs (Morgan, 1997; Muller *et al.*, 1996). Both inflammatory cytokines and nitric oxide may contribute to induction and progression of various disorders. For example, greater concentrations of nitric oxide have been linked to increased TNFα concentrations in congestive heart failure patients

(Comini *et al.*, 1999). Induction of nitric oxide by inflammatory cytokines may also play a role in chronic inflammatory bowel disorders (Perner and Rask-Madsen, 1999). Secretion of pro-inflammatory cytokines leading to increased production of nitric oxide contributes to neuronal tissue degeneration in neurological diseases such as multiple sclerosis and Alzheimer's disease (Heales *et al.*, 1999; Torreilles *et al.*, 1999). Therefore, it is not surprising that inhibition of nitric oxide synthase is currently being investigated as a potential therapeutic target (Hobbs *et al.*, 1999). Despite the detrimental effects of nitric oxide in septic shock and inflammatory disorders various nitric oxide donating drugs [e.g., nitroglycerine and sodium nitroprusside] are used clinically (Ignarro *et al.*, 1999; Murad, 1998). Nitric oxide donors are useful for diseases such as myocardial ischemia and treatment of acute hypertension refractory to standard drug therapy. The physiological actions of nitric oxide such as dilating arterial blood vessels, inhibiting platelet adherence and aggregation may be of benefit in these and perhaps other conditions (Lefer and Lefer, 1994). Therefore, nitric oxide has a role in both pathogenesis and treatment of disease and depending on the type of disease agents with either nitric oxide donating or inhibiting properties may be indicated.

Recent studies provide evidence that pro-inflammatory cytokines and inflammation markers: CRP, fibrinogen and erythrocyte sedimentation rate (ESR) are associated with cardiovascular disorders in humans (Verheggen *et al.*, 1999; Lagrand *et al.*, 1999). Increased concentrations of CRP are reported to be an independent risk factor for development of cardiovascular diseases (Lagrand *et al.*, 1999). Interestingly, increased CRP concentrations has been shown to be related to a family history of myocardial infarction (Margaglione *et al.*, 2000). It has been suggested that measuring

CRP concentrations in individuals at risk for developing cardiovascular disorders may be a useful predictor of impending disease (Rohde *et al.*, 1999). Increased expression of pro-inflammatory cytokines have been observed in various cardiovascular diseases such as congestive heart failure, unstable angina and post-myocardial infarction (Liuzzo *et al.*, 1999; Seta *et al.*, 1996; Irwin *et al.*, 1999; Guillen *et al.*, 1995). Interestingly, TNFα has a key role in control of body mass, and increased production causes cachexia, a wasting syndrome characterized by loss of muscle, fat and bone tissue which is observed in end-stage congestive heart failure (Anker *et al.*, 1999). Elevated concentrations of CRP and TNFα have also been observed in overweight and obese adults (Visser *et al.*, 1999; Dandona *et al.*, 1998). In contrast to cachexia, abnormalities in TNFα activity rather than production are suggested to be responsible for the lack of control of body weight (Argiles *et al.*, 1997). Secretion of pro-inflammatory cytokines from adipose tissue has been reported to cause a low grade inflammatory state in obese individuals reflected by increased concentrations of CRP, IL-6 and TNFα (Yudkin *et al.*, 1999). Overexpression of these inflammatory mediators in obese individuals was associated with insulin resistance and endothelial dysfunction.

Increased concentrations of pro-inflammatory cytokines are also associated with non-cardiovascular diseases. Here are few examples. Patients with various rheumatic diseases have been shown to have elevated concentrations of pro-inflammatory mediators (Kelley *et al.*, 1997). Interestingly, development of arthritis as a complication of $IFN_{\alpha 2a}$ treatment in individuals with various conditions has been reported (Nesher and Ruchlemer, 1998). Increased concentrations of TNFα and IL-1β are observed in patients suffering from Crohn's disease and ulcerative colitis (Nikolaus *et al.*, 1998). Greater

concentrations of TNFα and IL-6 are observed in schizophrenic patients compared to healthy individuals (Monteleone *et al.*, 1997; Naudin *et al.*, 1996). Increased concentration of IL-6, soluble IL-6 receptor (sIL-6r), soluble IL-2 receptor (sIL-2r), IL-1 ra, and transferrin receptor are reported for individuals with major depression (Maes *et al.*, 1995a; 1995b). In addition, $IFN_{\alpha 2a}$ administered to individuals with chronic hepatitis C caused depression in these patients (Malaguarnera *et al.*, 1998). Malaguarnera et al recommended a careful selection of hepatitis C patients before initiating $IFN_{\alpha 2a}$ therapy. Therefore, cytokines and markers of inflammation may be associated with various types of diseases.

Greater concentrations of pro-inflammatory cytokines and markers of inflammation occur not only in disease but are also reported for elderly individuals. Significantly, greater concentrations of TNFα and IL-6 were observed comparing healthy elderly individuals to those less than 65 years of age (Bruunsgaard *et al.*, 1999). An association between increased IL-6 concentrations and depression in older individuals has been reported (Dentino *et al.*, 1999). Interestingly, higher plasma levels of IL-6 may be used to predict disability onset in older people (Ferrucci *et* al., 1999). A recent study reported that high concentrations of CRP and IL-6 in healthy elderly individuals is associated with mortality (Harris *et al.*, 1999). Therefore, increased cytokine concentrations may occur not only with those having inflammatory conditions such as arthritis, Crohn's disease, psychiatric, and cardiovascular disorders but also in the elderly.

To treat patients with conditions characterized by elevated concentrations or pro-inflammatory cytokines, anti-cytokine therapy using specific antagonists of high affinity and specificity such as monoclonal antibodies, soluble cytokine inhibitors and anti-

inflammatory cytokines have been administered in clinical trials (Feldmann *et al.*, 1999). A few examples and results of anti-cytokine therapy and administration of anti-inflammatory cytokines will be discussed. TNFα regulates expression of pro-inflammatory mediators IL-1, IL-6 and IL-8 in rheumatoid arthritis (Feldmann *et al.*, 1996). Therefore, inhibiting TNFα would prevent an increased expression of pro-inflammatory cytokines in arthritic patients. Rheumatoid arthritic patients treated with anti-TNFα monoclonal antibodies are reported to have a reduced disease activity (Feldmann *et al.*, 1999). Similar to arthritis, overexpression of pro-inflammatory cytokines has an important role in the pathogenesis of heart failure (Seta *et al.*, 1996). Interestingly, administration of a specific TNFα antagonist to congestive heart failure patients resulted in significant improvements in functional status (Deswal *et al.*, 1999; Torre-Amione *et al.*, 1999).

Patients with moderate to severe Crohn's disease and Crohn's ileocolitis anti-TNFα was also beneficial (van Hogezand and Verspaget, 1998; Baert *et al.*, 1999). Secretion of TNFα and IL-1β in Crohn's and ulcerative colitis patients by polymorphonuclear neutrophil granulocytes were reduced by IL-10 administration which was thought to be due to a downregulation of macrophage and Th1 cell activity (Nikolaus *et al.*, 1998). In addition, administration of IL-10 to steroid refractory Crohn's patients resulted in either a favorable response or complete remission (van Deventer *et al.*, 1997). Interestingly, monoclonal antibodies that antagonize IL-2 receptor α-subunit administered to individuals receiving baseline immunosuppression therapy significantly reduced acute rejection after renal transplantation (Nashan *et al.*, 1999; Berard *et al.*, 1999). However, despite the success in clinical trials in treating arthritis, congestive heart

failure, Crohn's disease and preventing organ rejection cytokine neutralization i.e., anti-TNFα treatment failed to be of benefit in multiple sclerosis patients (Lenercept Multiple Sclerosis Study Group, 1999). In addition, a beneficial effect of IFN-β administration to patients with multiple sclerosis remains to be determined (Rice and Ebers, 1998; Tselis and Lisak, 1999). Similar to multiple sclerosis clinical trials of septic patients treated with anti-TNFα, soluble TNFα receptors, and IL-1ra have not demonstrated any beneficial effect (van der Poll and van Deventer, 1999). The ineffectiveness of these agents were suggested to be due to timing since patients admitted to the hospital were not in the systemic inflammatory response syndrome (SIRS) phase, which would respond to these agents. Instead, patients treated with these agents were in the phase referred to as compensatory anti-inflammatory response syndrome (CARS), which is characterized by anti-inflammatory cytokine release and subsequent refractory state (van der Poll and van Deventer, 1999; Groeneveld *et al.*, 1997). Therefore, pro-inflammatory cytokines play a role in disease occurrence and therapy. P-gp modulation, anti-cytokine agents and anti-inflammatory cytokines in the future may have a significant impact on pharmacotherapy of various diseases.

Inflammatory mediators and markers of inflammation have been associated with altered response to various drugs; here are some examples. Patients with unstable angina that were refractory to standard drug therapy were shown to have significantly higher concentrations of CRP, fibrinogen, and a greater ESR compared to patients that were stabilized (Verheggen *et al.*, 1999). Increased concentrations of IL-1ra and IL-6 in patients with unstable angina receiving standard drug therapy for the first two days of hospitalization were associated with increased risk of in-hospital coronary events

(Biasucci *et al.*, 1999). High CRP concentrations in myocardial infarction patients treated with thrombolytic drugs was shown to predict mortality up to 6 months after the infarction independent of drug treatment (Pietila *et al.*, 1996). Treatment resistant schizophrenia was associated with increased IL-6 concentrations (Lin *et al.*, 1998). Interestingly, effectiveness of clozapine in treating patients that are resistant to other anti-psychotic agents is suggested to be due to the complex immunomodulatory effects of the drug (Maes *et al.*, 1997a). In addition, increased serum IL-6 and IL-1ra concentrations were associated with major depression and treatment resistant depression (Maes *et al.*, 1997b). Therefore, inflammation and inflammatory mediators have not only an important role in host defense but also can induce disease, influence drug concentration and activity. It would be of value to determine whether administration of cytokine antagonists such as anti-TNFα would reverse the altered responsiveness to drugs observed in patients with inflammatory disease.

1.1.2 Drug disposition

Interest in inflammation-induced alterations in drug concentrations began when greater concentrations of propranolol were discovered serendipitously when investigating absorption of propranolol in patients with celiac disease (Schneider *et al.*, 1976). Patients with Crohn's disease and rheumatoid arthritis were initially studied then later patients with other inflammatory diseases such as ulcerative colitis, staphylococcal pneumonia, and systemic lupus erythromatosus were included (Schneider *et al.*, 1981). Interestingly, greater plasma concentrations of oxprenolol were detected in patients with viral illness (Kendall *et al.*, 1979). The mechanisms responsible for increased propranolol and

oxprenolol concentrations were unclear, however, the only variable that seemed to be associated was an increased ESR a nonspecific marker of inflammation. Possible explanations for these increased drug concentrations may involve alterations in drug absorption, distribution and metabolism.

1.1.2.1 Absorption

It has been suggested that alterations in drug absorption may account for the elevated concentrations of drug measured in inflammatory disease (Cooper and Lucas, 1976). Although there are reports indicating that disease may alter intestinal integrity and influence absorption of drugs (Gibaldi, 1991), changes in drug absorption have not been proven to be the cause of increased drug concentrations in patients with inflammatory disease. Kirch et al in 1983 reported a reduced absorption of atenolol in patients with acute respiratory tract infection and an ESR>20 mm/1h. However, no significant differences in total urinary recovery of atenolol were observed between healthy and inflamed groups. Therefore, this observation requires confirmation. Although differences in gastric emptying may influence peak drug concentration no relationship was found between maximum concentration of propranolol and administration of metoclopramide and propantheline which stimulate and depress gastric emptying respectively (Castleden *et al.*, 1978). In addition, propranolol is a weak base with a pKa of 9.45 and according to the theory of diffusion propranolol should be less ionized and better absorbed in an alkaline environment (Gibaldi, 1991). Thus, perhaps an increase in intestinal pH occurs with inflammatory disease that may cause a greater drug concentration. However, the

increase in pH of the luminal surface of the upper jejunum where the majority of propranolol is absorbed was found to be only marginally increased from pH of 5.9 to 6.4 in patients with Crohn's disease. Propranolol concentrations were also greater in Crohn's disease than celiac disease in which a higher intestinal pH of 7.0 was measured (Lucas *et al.*, 1976; Schneider *et al.*, 1979). Increased absorption of drug in individuals with inflammation is also not likely an explanation since propranolol and oxprenolol are almost completely absorbed from the intestine (propranolol > 90% and oxprenolol 70-95%) in healthy individuals (Frishman, 1979). In addition, absorption of propranolol from the upper jejunum in rats with adjuvant arthritis was reported not to be influenced by concomitant inflammatory disease (Key *et al.*, 1986). Therefore, absorption of drug in laboratory animals and patients with inflammatory disease does not appear to be altered consequently does not cause the increased drug concentrations.

1.1.2.2 Distribution

In arthritis and inflammatory disease increased expression of pro-inflammatory cytokines [i.e., TNFα, IL-1 and IL-6] are observed (Bondeson, 1997; Feldmann *et al.*, 1996). These inflammatory mediators i.e., IL-6 may then act on the liver to increase production of acute phase reactant proteins such as AAG that binds to basic drugs (De Leve and Piafsky, 1981). Piafsky et al in 1978 found greater concentrations of AAG in patients with Crohn's disease and inflammatory arthritis. In addition, a strong negative correlation between percentage of unbound propranolol and concentration of AAG existed thus greater AAG concentrations resulted in reduced free fraction of propranolol

(Piafsky *et al.*, 1978). A reduced albumin concentration and weak but positive correlation between albumin concentration and free fraction of propranolol was also observed. Despite reduced albumin concentrations propranolol binding was greater in inflammation since propranolol is a basic drug mainly bound to AAG (Belpaire *et al.*, 1982).

It has been suggested that increased protein binding rather than changes in metabolism or hepatic blood flow may be responsible for the altered drug concentration observed in laboratory animals and humans with inflammatory diseases (Walker *et al.*, 1986). Intravenous administration of IL-1β to rats dosed orally with propranolol resulted in an enantioselective increase in plasma drug concentration (Vermeulen *et al.*, 1993). In the cytokine treated rats the AUC of R-propranolol was significantly higher than S-propranolol. The mechanism responsible for the enantioselective increase was suggested to be protein binding since plasma protein binding was significantly greater for the R-enantiomer. In addition, the effect of endotoxin-induced inflammation on enantioselective pharmacokinetics of verapamil, propranolol and oxprenolol in the rat were studied (Laethem *et al.*, 1994). It was concluded that preferential increase of R-oxprenolol, R-propranolol and S-verapamil in the endotoxin treated rat was due mainly to an enantioselective increase in binding of the enantiomers to AAG.

However, changes in protein binding could not account for the increase in plasma levels of propranolol, metoprolol, and antipyrine in rats with turpentine-induced inflammation (Belpaire *et al.*, 1989). In the turpentine treated rat, free fraction of propranolol after both intravenous and oral administration was reduced. In addition, after intravenous dosing of propranolol a reduced volume of distribution, perhaps, due to increased binding to AAG and a decreased systemic clearance was reported. Since

propranolol is highly extracted by the liver according to the well-stirred model systemic clearance is dependent on hepatic blood flow (Wilkinson and Shand, 1975). Therefore, the reduced systemic clearance after intravenous administration of propranolol suggests a reduced hepatic blood flow in the arthritic rat. Interestingly, after intravenous administration of metoprolol, a highly extracted negligibly protein bound drug, no differences were observed in systemic clearance between healthy and arthritic rats. However, after oral administration of metoprolol a decreased clearance resulting in a greater AUC in the turpentine treated rat was detected. Protein binding of metoprolol is negligible and was not altered therefore the greater AUC after oral administration in the inflamed rat may be explained by a reduction in intrinsic clearance. Since with highly extracted drugs a decrease in intrinsic clearance is observed by a decrease in presystemic rather than systemic clearance (Gibaldi and Perrier, 1982). Turpentine-induced arthritis did not alter systemic clearance of metoprolol, this indicates that hepatic blood flow is not significantly altered by the inflammation. This suggests the greater propranolol AUC after intravenous administration to the arthritic rat is likely due to increased protein binding. Interestingly, antipyrine was given intravenous to healthy and turpentine inoculated rats. The half-life of antipyrine increased with no changes in volume of distribution and a reduced systemic clearance was also observed. Since antipyrine is a low extraction drug thus dependent on protein binding and intrinsic hepatic clearance (Wilkinson and Shand, 1975). The reduction in systemic clearance is due to a reduced intrinsic clearance since antipyrine binds negligibly to plasma proteins. Therefore, the effect of turpentine-induced arthritis on propranolol, metoprolol and antipyrine

pharmacokinetics suggests inflammation induced alterations in drug disposition are due to a combination of altered protein binding and reduced metabolism.

Propranolol and other highly extracted drugs have been shown to undergo nonrestrictive protein binding. Thus, elimination is not limited by binding to plasma proteins (Shand *et al.*, 1976; Gariepy *et al.*, 1992). Therefore, increased binding to AAG should not protect drug passing through the liver. In fact, the increased binding to AAG may even facilitate uptake of propranolol by the liver. However, intrinsic clearance may be decreased to such an extent that propranolol is no longer considered a highly extracted drug (Belpaire *et al.*, 1989). In addition, this property of protein binding may not exist or be different in inflammatory diseases therefore requires further investigation (Schneider *et al.*, 1981).

1.1.2.3 Metabolism

Inflammatory disease and pro-inflammatory cytokines have been shown to alter drug metabolism. A marked reduction in N-demethylase, $NADPH_2$-oxidase and CYP activity has been observed in laboratory animals that have inflammation (Morton and Chatfield 1970; Cawthorne *et al.*, 1976; Whitehouse, 1973). In addition to phase I drug metabolism formation of β-glucuronide and sulfate conjugates have been observed to be reduced by inflammatory diseases. For example, Morton and Chatfield 1970 reported diminished excretion of acetaminophen conjugates in rats with adjuvant arthritis.

A significant increase in plasma concentrations of acebutolol, a cardioselective β-adrenergic blocker that has limited protein binding (7-12%), was observed in adjuvant

arthritic rats (Piquette-Miller and Jamali, 1992). Acebutolol is an intermediate extraction drug with an extraction ratio of 0.65 thus hepatic extraction is dependent on protein binding, intrinsic clearance, and hepatic blood flow. Since protein binding of acebutolol is negligible and was not altered by inflammation and hepatic blood flow is reportedly unchanged in adjuvant arthritis (Walker *et al.*, 1986). This suggests the greater concentrations of acebutolol enantiomers in the arthritic rat resulted from reduced intrinsic hepatic clearance of drug.

Interestingly, altered drug metabolism in inflammatory disease has been attributed to pro-inflammatory cytokines inducing formation of nitric oxide (Morgan, 1997). Nitric oxide and breakdown products such as peroxynitrite are considered to bind to the heme group and irreversibly modify CYP activity (Morgan, 1997; Muller *et al.*, 1996). Therefore, increased expression of pro-inflammatory cytokines and subsequent formation of nitric oxide plays a role in the altered metabolism of drug observed in laboratory animals and humans with inflammation.

1.1.2.4 Renal elimination

Although abnormalities of renal function may be observed due to disease and/or therapy in patients with inflammation (Kelley *et al.*, 1997), there are few reports in the literature that investigates the effect of inflammation on renal clearance of drugs. One study reported that inflammatory bowel disease had no effect on plasma concentrations of renally excreted β-adrenergic blocker practolol (Parsons *et al.*, 1976). However, more studies on the effect of inflammation on drugs cleared via the renal route are required.

1.1.3 Disease severity and management

A combination of altered binding to plasma proteins, hepatic intrinsic clearance, and possibly, P-gp expression may be responsible for the increased concentration of drug observed in laboratory animals and patients with inflammatory disease. However, differences in disease activity exist between different species. For example, metoprolol concentrations were increased four fold in turpentine-induced arthritic rats (Belpaire *et al.*, 1989), however, inflammatory disease failed to alter metoprolol disposition in humans (Schneider *et al.*, 1981). In addition, in experimental models of inflammation such as adjuvant arthritis, extensive joint involvement occurs while patients with arthritis may have minimal joint involvement (Myles Glenn *et al.*, 1977). Schneider et al in 1979 grouped patients with Crohn's disease and rheumatoid arthritis into two groups depending on the ESR. Patients with an ESR>20 mm/1h had higher propranolol concentrations than those with an ESR<20 mm/1h (Schneider *et al.*, 1979). However, these studies were not quantitative, thus effect of inflammation severity on degree of increased drug concentration could not be determined. Influence of severity of inflammatory disease on drug levels was confirmed by Piquette-Miller and Jamali who reported that a progressively rising AUC of propranolol enantiomers in the arthritic rat was correlated with increasing disease activity (Piquette-Miller and Jamali, 1995). Nonsteroidal anti-inflammatory drugs (NSAIDs) i.e., ketoprofen treatment reduced the severity of inflammation and normalized plasma concentrations of propranolol stereoisomers in rats with adjuvant arthritis (Piquette-Miller and Jamali, 1995). Interestingly, despite normalization of propranolol pharmacokinetics by ketoprofen treatment prolongation of the cardiac PR interval was still less in the rat with adjuvant

arthritis compared to controls (Guirguis and Jamali, 1996), indicating reduced β-adrenergic responsiveness. Therefore, severity of inflammation influences drug disposition and response to drug may still be reduced despite normalization of plasma concentrations by NSAID treatment.

Antihypertensive drugs and NSAIDs are extensively used to treat cardiovascular and various types of inflammatory disorders respectively (Johnson, 1997; Fierro-Carrion and Ram, 1997). With this in mind, many individuals are concurrently taking NSAIDs and antihypertensive drugs. Administration of NSAIDs have a significant effect on blood pressure control in normotensive and hypertensive individuals (Ruoff, 1998). This is especially relevant to older individuals since NSAID and antihypertensive medication use increases with age to greater than 50 % in elderly individuals (Johnson, 1998). NSAIDs may inhibit two isoforms of cyclooxygenase (COX) enzymes to reduce inflammation that are COX-1 and COX-2 which have differing activities (Donnelly and Hawkey, 1997). COX-1 is involved in the normal functioning of the gastrointestinal tract therefore is constitutive and involved in normal physiological functions. However, COX-2 is induced by various inflammatory mediators and is involved in the acute inflammatory response. Interference of blood pressure control by NSAIDs is considered to be due to nonspecific inhibition of COX enzymes preventing formation of endoperoxide prostaglandins PGG_2 and PGH_2 from arachidonic acid. These endoperoxides are metabolized to a variety of eicosanoids including prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) and thromboxane A_2 (Ruoff, 1998). Blocking the formation of PGE_2 and PGI_2 with NSAIDs has a significant impact blood pressure control since these two prostaglandins are important vasodilators in the kidney (Dunn and Hood, 1977). In addition, PGE_2 inhibits the renal

absorption of salt and reabsorption of water thus reducing salt and water retention (Whelton, 1999).

NSAIDs may alter responsiveness to cardiovascular drug therapy via inducing gastrointestinal ulceration and inflammation (Guirguis and Jamali, 1999). Treatment with flurbiprofen resulted in significant changes in electrocardiographic PR interval, hence, altered β-adrenergic activity. However, co-treatment with metronidazole, an antimicrobial and free radical scavenger, prevented PR interval changes observed when administering flurbiprofen. In support of this observation, nitric oxide donor compounds and free radical scavengers used concomitantly with NSAIDs are reported to protect the gastrointestinal tract from ulceration (Davies and Jamali, 1997). Interestingly, nitric oxide donating ASA, while inhibiting both COX-1 and 2 did not alter the healing response of gastric ulcers while NS-398 which is a selective COX-2 inhibitor impaired healing of gastric ulcers (Ukawa *et al.*, 1998). Therefore, responsiveness to antihypertensive drugs when NSAIDs are co-administered may not be problematic if inflammation caused by the NSAID-induced ulceration is prevented. Perhaps, use of NSAIDs that has nitric oxide donor groups and scavenges free radicals or selective COX-2 inhibitors, if ulceration is not present, will keep the GI tract healthy and allow for adequate blood pressure control.

1.1.4 Drug activity

Despite concern over greater concentrations of propranolol and other cardiovascular drugs in laboratory animals and humans with inflammatory disease there are few reports in the literature reporting clinical outcomes (Schneider and Bishop, 1982).

This is alarming since these increased drug concentrations suggest enhanced effect or possibly toxicity. To examine the effect of inflammation on drug activity *in vitro* and *in vivo* studies will be discussed.

1.1.4.1 In vitro

Pro-inflammatory mediators have been shown *in vitro* to reduce activity of β-adrenergic receptors (Lui *et al.*, 1999; Gulick *et al.*, 1989). Lui et al measured responsiveness of β-adrenergic receptors to isoproterenol in the presence and absence of IL-1β in adult rat ventricular myocytes. Decreased β-adrenergic response to isoproterenol was observed in myocytes incubated with IL-1β compared to controls. The altered response in the presence of the pro-inflammatory cytokine was not affected by administration of either cAMP or cAMP donors thus it was concluded that reduced β-adrenergic response was due to a cAMP-independent mechanisms. However, Gulick et al cultured rat cardiac myocytes in the presence of activated immune cell cultures in which the presence of IL-1β and TNFα was confirmed. Administering isoproterenol a reduction in cardiac contractile responsiveness to β-adrenergic stimulation was observed in the cells incubated with inflammatory mediators compared to controls. Intracellular cAMP was reduced in the myocytes exposed to the inflammatory cytokines, therefore, the decreased β-adrenergic response was considered cAMP-dependent. Results from these two studies are contradictory and indicate that the mechanism(s) responsible for β-adrenergic downregulation in the presence of inflammatory mediators remains to be determined.

Altered responsiveness of cardiovascular receptors *in vitro* has also been shown to be associated with advancing age. Ventricular biopsies from non-failing ventricles of young and old individuals undergoing cardiac bypass surgery were subjected to isoproterenol stimulation (Davies *et al.*, 1996). A decreased responsiveness of β-adrenergic receptors was observed in biopsies from older individuals, this was suggested to be due to a reduction in β-adrenoceptor density with age. Age-associated changes in β-adrenergic receptors were also investigated using rat ventricular myocytes from rats that were 2, 8, and 24 months of age (Xiao *et al.*, 1998). A significant depression in contractile response to isoproterenol was observed in myocytes from rats 24 month old which was attributed to a decreased receptor density and membrane adenylate cyclase activity. Thus, *in vitro* studies have shown that aging may cause altered responsiveness of β-adrenergic receptors.

Cytokines have also been shown to modulate β-adrenergic control of cardiac myocytes through production of nitric oxide. Cardiac pig myocytes exposed to IL-1β stimulated with isoproterenol had significantly less β-adrenergic control of cardiac calcium current than controls (Rozanski and Witt, 1994). Addition of IL-1ra to the myocytes, substituting D-arginine for L-arginine, and incubating the myocytes with a nitric oxide inhibitor N^G-monomethyl-L-arginine (L-NMMA) prevented the altered β-adrenergic response in the presence of IL-1. Thus, it was concluded that IL-1 influences β-adrenergic control of cardiac Ca^{2+} channels by mechanisms that involve nitric oxide. In addition, involvement of nitric oxide in ventricular myocyte contractility from failing and non-failing hearts was determined (Flesch *et al.*, 1999). Treatment of the myocytes with endotoxin resulted in decreased isoprenaline-induced contractions. Also, administration

of nitric oxide synthase inhibitor L-NMMA resulted in greater isoprenaline-induced contractions thus an increased responsiveness of β-adrenergic receptors was observed. In this study, incubating the myocytes with Trion, a superoxide and peroxynitrite scavenger, abolished the effect of endotoxin on responsiveness to isoprenaline. Therefore, impaired myocardial contraction was suggested to be due to endotoxin exposure and increased expression of nitric oxide and peroxynitrite. Another recent study reported that the effect of endotoxin on cultured rat cardiomyocytes resulted in greater concentrations of nitric oxide, IL-6, IL-1 which influenced β-adrenoceptor mediated function but not α-adrenoceptor activity (Muller-Werdan *et al.*, 1998). In the presence of dexamethasone, an inhibitor of inducible nitric oxide synthase, the endotoxin-mediated reduction of β-adrenoceptor activity was inhibited. However, TNFα in this investigation depressed both β and α-adrenoceptor function in the presence and absence of dexamethasone thus in a nitric oxide independent manner. This indicates that altered cardiovascular receptor responsiveness *in vitro* may also be observed independent of nitric oxide production.

A reduced cardiac calcium channel responsiveness of rat cardiomyocytes in the presence of inflammatory mediators *in vitro* has also been reported (Krown *et al.*, 1995; Liu and Schreur, 1995). Exposure of cardiac myocytes to TNFα and IL-1β resulted in a suppression of cardiac L-type calcium channels which was suggested to be due to an alteration in signaling pathways responsible for calcium channel function. In addition, ventricular myocytes from guinea pigs treated with LPS exhibited a reduced L-type cardiac calcium current (Zhong *et al.*, 1997). It was concluded that a decreased L-type calcium current plays a central role in myocardial contractile dysfunction observed during endotoxemia.

Administration of $IFN_{\alpha 2a}$ to rabbit ventricular myocytes reduced *in vitro* ATP-sensitive potassium channel activity (Nishio *et al.*, 1999). Reduced activity of cardiac potassium channels in the inflammatory state has also been shown using ventricular myocytes from rats that had undergone an experimentally induced myocardial infarction (Kaprielian *et al.*, 1999). An altered function or downregulation of potassium channels was reported which was thought to be due to reduced potassium channel density. Pro-inflammatory cytokine concentrations were not measured which is unfortunate since enhanced inflammatory cytokine expression after myocardial infarction has been reported (Neumann *et al.*, 1995; Guillen *et al.*, 1995; Irwin *et al.*, 1999). Perhaps, increased expression of inflammatory mediators may have been associated with reduced cardiac potassium channel function. Therefore, the *in vitro* studies discussed indicate a reduced β-adrenergic, cardiac calcium, and potassium channel receptor function in the presence of inflammatory disorders and pro-inflammatory cytokines.

1.1.4.2 In vivo

One of the first reports of altered response to cardiovascular drug in laboratory animals was by Belpaire et al in 1986. After intravenous administration of propranolol to rats with turpentine induced inflammation a significantly higher plasma concentration of propranolol was observed. Despite greater propranolol concentrations in rats with inflammation, surprisingly, in the arthritic rat, propranolol had no effect on isoproterenol induced tachycardia. However, propranolol reduced the effect of isoproterenol on heart rate by approximately 75 % in healthy rats. The reduced β-adrenergic response to

propranolol in the rat with arthritis was attributed to increased protein binding, hence, less unbound propranolol was able to interact with the β-adrenergic receptor. In addition, increased propranolol concentrations and decreased β-adrenergic responsiveness to propranolol in the adjuvant arthritic rat is reported (Guirguis and Jamali, 1996). Inflammation caused by $IFN_{\alpha 2a}$ inoculation has been shown to reduce responsiveness of cardiac calcium channels to verapamil and was associated with increased concentrations of nitric oxide (Mayo *et al.*, 1996).

Alteration in receptor response to drug due to enhanced cytokine concentrations may not only be limited to inflammatory diseases but may also occur in the elderly. Decreased prolongation of the PR interval, indicating reduced sensitivity of cardiac calcium channels to verapamil has been reported in older patients compared to younger individuals (Abernethy *et al.*, 1986; 1993). This, perhaps, is due to an increased expression of inflammatory cytokines in the aged affecting cardiac calcium channel activity. In addition, altered adrenergic sensitivity to β-blockers has also been shown to occur with advancing age as seen with propranolol (Tenero *et al.*, 1990; Brodde *et al.*, 1995). Clinically, therefore, β-adrenergic receptor and cardiac calcium channel downregulation may occur in various circumstances where increased pro-inflammatory cytokine concentrations are observed. For example, in patients with inflammatory conditions such as arthritis, Crohn's disease, infection and also in the elderly.

Reduced responsiveness to cardiovascular drug has been observed in patients with various cardiovascular diseases. Inflammatory status has been shown to determine the clinical course of post myocardial infarction patients who have unstable angina and receive standard drug therapy (Verheggen *et al.*, 1999). It has been suggested that

indiscriminately treating patients with unstable angina regardless of inflammatory state may not be appropriate (Liuzzo *et al.*, 1999). In addition, increasing levels of IL-1ra and IL-6 during the first 2 days of hospitalization in patients with unstable angina were associated with increased risk of in-hospital coronary events in patients receiving standard drug therapy (Biasucci *et al.*, 1999). The relationship between serum CRP concentration after acute myocardial infarction and 24 month survival was investigated. Patients with significantly higher CRP concentrations in the first few days after an acute myocardial infarction treated with thrombolytic drugs had an increased risk of death during the first 6 months after the infarction (Pietila *et al.*, 1996). Therefore, inflammatory conditions or inflammation-induced pathophysiological changes can decrease responsiveness to cardiovascular drugs.

1.2 Sotalol

1.2.1 Indications

Sotalol is a racemic non-selective, β-adrenergic antagonist/cardiac potassium blocker, and is indicated for the treatment of hypertension, angina, and ventricular arrhythmias (Anderson and Prystowsky, 1999). Sotalol has no intrinsic sympathomimetic or membrane stabilizing activities and elicits its antihypertensive/antiarrhythmic effect by non-selectively blocking β-adrenergic receptors and potassium channels in the myocardium (Anderson and Prystowsky, 1999). Sotalol was first synthesized in 1960 and

marketing began in 1974, therefore, racemic sotalol has been used as an antihypertensive and antianginal drug for more than 25 years.

The antiarrhythmic activity of sotalol has resulted in renewed interest in the drug since trials such as the CAST (Cardiac Arrhythmia Suppression Trial) proved an increased risk of sudden death with class I antiarrhythmic agents (Bauman, 1997). As a result of the CAST findings class I antiarrhythmic agents have come under scrutiny. Sotalol inhibits the delayed rectifier and other cardiac potassium channels, in 1992 the racemate was approved by the Food and Drug Administration for use in the treatment of life-threatening ventricular arrhythmias (Anderson and Prystowsky, 1999). Interestingly, potassium channel blockers have been suggested to reduce mortality caused by left ventricular dysfunction after myocardial infarction (Waldo *et al.*, 1996). Therefore, to determine whether S-sotalol, potassium channel antagonist, reduces mortality in patients with previous myocardial infarction and left ventricular dysfunction the Survival With Oral d-sotalol (SWORD) trial was conduced. This trial was stopped before completion due to a higher mortality in the sotalol treated group (Waldo *et al.*, 1996). A possible reason for the increased mortality with S-sotalol, perhaps, is that β-adrenergic blockade is required to counteract the sympathetic activation that accompanies prolongation of the cardiac action potential (Schwartz, 1998). Despite cancellation of the SWORD study there is renewed interest in S-sotalol as an agent to treat sustained ventricular tachycardia (Advani and Singh, 1995). Also, use of S-sotalol to decrease defibrillator energy requirements in patients with an automatic implanted cardioverter-defibrillator (AICD) is gaining interest due to the increased safety margin since lower energies for defibrillation are required (Dorian *et al.*, 1996).

1.2.2 Physiochemical properties

The chemical structure of sotalol ($C_{12}H_{20}N_2O_3S$) is depicted in Figure 1-1. Sotalol is an odorless white crystalline powder (Budavari, 1996; Reynolds, 1996). The molecular weight of the base and hydrochloride (HCl) salt are 272.37 and 308.83 respectively. Sotalol is a hydrophilic β-adrenergic blocker with a water/*n*-octanol partition coefficient (log p value) of 0.24 (Burgot *et al.*, 1990). Using the octanol/phosphate buffer (pH=7.4) at 37° C, sotalol was reported to have a partition coefficient of 0.09 (Jack *et al.*, 1988).

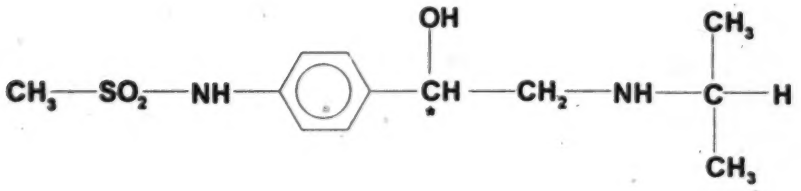

Figure 1-1. Chemical structure of sotalol, * denotes chiral center.

Unlike other β-adrenergic antagonists that are aryloxypropranolamines, sotalol enantiomers are methanesulfonamide-substituted phenethanolamines thus are amphoteric. Therefore, the pKa values are 9.8 and 8.3 for the amine and sulfonamide respectively (Doerge, 1982).

A Perkin Elmer model 241 spectrophotometer was used to obtain the optical rotations of R and S-sotalol. The rotations were measured in a 10 cm (centimeter) cell

and using water as a solvent at the sodium D-line (589 nm). The optical rotations or specific rotations of sotalol hydrochloride enantiomers were:

(+)-(S)-sotalol hydrochloride $[\alpha]^{25}_{D}$	+35.80°
(−)-(R)-sotalol hydrochloride $[\alpha]^{25}_{D}$	−34.75°

The specific rotations of sotalol hydrochloride in methanol were reported (Le-Garrec *et al.*, 1987) as:

(+)-(S)-sotalol hydrochloride $[\alpha]^{25}_{D}$	+39.90°
(−)-(R)-sotalol hydrochloride $[\alpha]^{25}_{D}$	−36.30°

The melting point of racemic sotalol has been reported within the range of 206.5-207° C (Doerge *et al.*, 1982).

1.2.3 Pharmacokinetics-pharmacodynamics

The oral bioavailability of sotalol racemate is 90-100% in humans (Anderson and Prystowsky, 1999). The absorption rate is less than other β-adrenergic antagonists with peak concentrations occurring at 2 to 4 h (Singh *et al.*, 1987). Sotalol does not undergo first-pass metabolism and is mainly eliminated unchanged via the renal route by glomerular filtration and tubular secretion (Singh *et al.*, 1987). In humans protein binding

is reported to be less than 2 % with albumin and AAG contributing to the binding (Belpaire *et al.*, 1982). Sotalol is excreted in breast milk and the milk:serum ratios ranged from 2.43 to 5.64 (Hackett *et al.*, 1990). Diseases that alter renal excretion of sotalol will generally alter pharmacokinetics and pharmacodynamics as shown by the increased β-adrenergic and cardiac potassium channel blocking that may produce toxicity in patients with renal failure (Singh *et al.*, 1987).

The concentration effect relationships used to model the activities of sotalol are maximum effect (E_{max}) and sigmoid E_{max} models (Singh *et al.*, 1987).

1.3 Lidocaine

1.3.1 Indications

Lidocaine was first used as an antiarrhythmic agent in 1950 and is a drug of choice for the acute management of ventricular arrhythmia associated with myocardial infarction, surgery, and ventricular tachycardia (Alexander *et al.*, 1998). The use of lidocaine for routine prophylaxis of acute myocardial infarction is not recommended since survival was reported to be reduced due to exacerbation of heart block and congestive heart failure (Roden, 1996). Lidocaine elicits its therapeutic effect via blocking cardiac fast sodium channels and is effective against ventricular arrhythmias of a diverse etiology (Alexander *et al.*, 1998). Since rapid onset of drug occurs due to intravenous administration and rapid clearance the effects of lidocaine decrease quickly upon termination of administration permitting titration of the dose for ectopic activities.

Therefore, due to the rapid clearance and ease of titration of dose, lidocaine is still a popular antiarrhythmic agent (McEvoy, 1999). However, clinical monitoring is essential due to narrow therapeutic index and high intersubject variability (Pieper and Johnson, 1992). Therefore, plasma concentrations of lidocaine are monitored and adjusted to prevent hemodynamic and other complications. Lidocaine is well tolerated at therapeutic concentrations 1.5 to 5.5 μg/ml for arrhythmia suppression (Rodighiero, 1989).

1.3.2 Physiochemical properties

The chemical structures of lidocaine ($C_{14}H_{22}N_20$) and monoethlyglycinexylidide (MEGX) ($C_{12}H_{18}N_2O$) are depicted in Figures 1-2 and 1-3 respectively. Lidocaine is a white odorless crystalline powder (Budavari, 1996; Reynolds, 1996). The molecular weights of the base and HCl salt are 234.33 and 270.79 respectively (Reynolds, 1996). Lidocaine is practically insoluble in water, however, the HCl is very soluble in water and alcohol. Using a $CHCl_3$/phosphate buffer (pH=8.0) at 23° C lidocaine was reported to have a partition coefficient of 99 (Nyberg *et al.*, 1977).

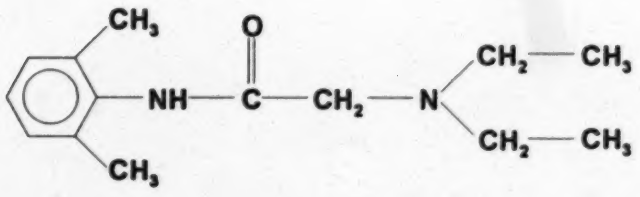

Figure 1-2. Chemical structure of lidocaine.

Lidocaine is a weak base with a pKa of 7.85 and exists predominantly as the protonated form at physiological pH of 7.4 and melting points of lidocaine crystals are 127 to 129° C (Budavari, 1996).

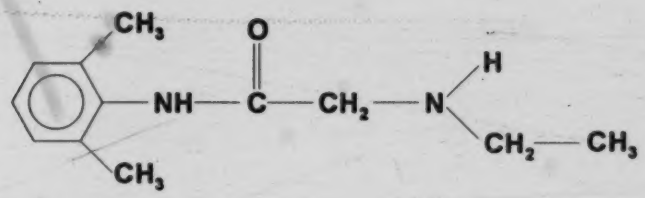

Figure 1-3. Chemical structure of MEGX.

1.3.3 Pharmacokinetics-pharmacodynamics

Lidocaine HCl is absorbed well from the gastrointestinal tract, however, due to extensive and highly variable first pass metabolism only 35 ± 13% of an oral dose reaches the systemic circulation (Pieper and Johnson, 1992). This high variability after oral administration to humans is due to the large variation i.e., 4 to 13 fold in CYP3A expression (de Wildt *et al.*, 1999). In addition, one study reported that therapeutic plasma concentrations of lidocaine were not obtained after oral administration of 250 mg and 500 mg but toxic effects occurred, perhaps, due to high concentrations of toxic metabolites (McEvoy, 1999). Therefore, due to the condition of the patient, requirement for rapid

dosage adjustment and increased production of toxic metabolites lidocaine is not administered orally. Lidocaine is widely distributed into body tissues and has a high affinity for fat and adipose tissue. Binding of lidocaine to plasma proteins is variable and concentration dependent. In the therapeutic range, lidocaine is approximately 70 % bound to plasma proteins. The majority of lidocaine i.e., 70 % is bound to AAG and remaining 30 % to albumin (Pieper and Johnson, 1992). At therapeutic concentrations, the ratio of blood-to-plasma concentrations is 0.8 and 0.9 in healthy volunteers (Pieper and Johnson, 1992). Lidocaine is distributed into breast milk and concentration has been reported to be as high as 40 % of serum from a sample taken approximately 2 h after lidocaine administration (McEvoy, 1999).

Lidocaine is eliminated predominantly by hepatic metabolism in both humans and rats (Imaoka *et al.*, 1990; Pieper and Johnson, 1992). There is no evidence of biliary secretion in humans and renal clearance of lidocaine is considered insignificant since less than 10 % of an intravenous dose is excreted unchanged in the urine (Pieper and Johnson, 1992). The hepatic extraction ratio in man ranges from 0.62 to 0.81 (Pieper and Johnson, 1992). Phase I metabolism of lidocaine is extensive and occurs at the tertiary amine (N-dealkylation) at the para- or meta- positions of the aromatic ring (hydroxylation) and at the arylmethyl group (hydroxylation) and at the amide linkage (hydrolysis). This results in formation of MEGX, 3- or 4-hydroxy lidocaine, methylhydroxy lidocaine, and 2,6-xylidine respectively (Beckett *et al.*, 1966; Nelson *et al.*, 1977; Suzuki *et al.*, 1984). The glycine nitrogen is susceptible to N-oxidation by rat liver microsomes (Patterson *et al.*, 1986).

The metabolites MEGX, 3-, 4-hydroxy lidocaine, methylhydroxy lidocaine and 2,6, xylidine may also undergo secondary N-dealkylation, ring hydroxylation, amide hydrolysis and conjugation. The conjugation reactions are mainly glucuronidation and sulfation and N-conjugation of the aromatic amine is negligible in rats and humans (Keenaghan and Boyes, 1972; Kawai *et al.*, 1985).

In humans, 74-80 % of lidocaine is recovered as the conjugates of xylidide and 4-hydroxy-xylidide (Keenaghan and Boyes, 1972). This indicates N-dealkylation and cleavage of the amide bond of lidocaine and metabolites are predominant routes of biotransformation (Nelson *et al.*, 1977; Hollunger, 1960). The major metabolite of lidocaine MEGX is predominantly eliminated by the liver and follows formation rate-limited kinetics and has 83 % activity of lidocaine (Burney *et al.*, 1974; Strong *et al.*, 1975; Rodighiero, 1989). In humans, N-deethylation of lidocaine to MEGX is catalyzed by CYP3A4 (Bargetzi *et al.*, 1989; Imaoka *et al.*, 1990).

There are numerous reports on species, strain, gender, and age differences regarding lidocaine hepatic metabolism (Masubuchi *et al.*, 1991; Imaoka *et al.*, 1990; Fujita *et al.*, 1985; Lennard *et al.*, 1983). The comparison of humans to rats will be discussed. Lidocaine is extensively metabolized thus is classified a high extraction drug with systemic clearance dependent on hepatic blood flow in both humans and rats after intravenous administration (Keenaghan and Boyes, 1972). In humans, the dominant pathway is N-dealkylation followed by secondary N-dealkylation and then amide hydrolysis. In the rat there are two sites competing for lidocaine, high affinity low capacity site in which the 3-hydroxy metabolite is formed and a low affinity high capacity site in which N-dealkylation occurs (von Bahr *et al.*, 1977). Therefore, at low

lidocaine concentrations 3-hydroxy lidocaine is produced, however, at higher concentrations of lidocaine MEGX is 90 % of the total metabolites. In the rat substrate specificity of CYP3A overlaps with CYP2C (Smith, 1991). In humans, CYP3A4 catalyzes N-dealklyation of lidocaine to MEGX and CYP2C S-mephenytoin hydroxylation. This is reversed in the rat as MEGX formation is mainly catalyzed by CYP2C11 and mephenytoin hydroxylation by CYP3A (Smith, 1991; Masubuchi *et al.*, 1993). Rat CYP3A2 has only a small contribution to N-dealkylation activities. Therefore, lidocaine in the rat is metabolized mainly by CYP2C11 to MEGX with CYP1A2, 2B1 and 3A2 playing a minor role. Hydroxylation of aryl methyl group is catalyzed by CYP2B2 in rat microsomes (Imaoka *et al.*, 1990; Oda *et al.*, 1989). In the rat formation of 3-hydroxy lidocaine is catalyzed by CYP1A2 and 2D1/2 (Nakamoto *et al.*, 1997; Masubuchi *et al.*, 1991; 1993). The position of ring hydroxylation of lidocaine is species dependent. In man, hydroxylation selectively occurs at the para-positions but in rats at the meta-position. No gender differences are observed in humans (Smith, 1991). In addition, gender differences exist with preferential expression of CYP2C11 and 3A2 in male but not female rats (Smith, 1991).

Lidocaine is classified by the Vaughn-Williams classification of antiarrhythmic drugs as a class IB agent (McEvoy, 1999). A membrane stabilizer that reduces the refractory period by interacting with cardiac sodium channels in the open and inactivate state to inhibit recovery after repolarization (McEvoy, 1999; Roden, 1996). Recovery from lidocaine block is rapid so lidocaine exerts greatest effect on partially depolarized and/or rapid driven tissues (Nolan, 1997). The site of action in the heart is Purkinjie fibers, ventricular muscle and ventricular ectopic foci (Roden, 1996). The responsiveness

of ischemic tissue to lidocaine tends to be greater when compared to the normal myocardium. The sinoartrial (SA) and atrioventricular (AV) nodes and atrial muscle and ectopic foci are generally unresponsive to lidocaine in humans (McEvoy, 1999). At therapeutic concentrations automaticity of ventricular muscle is suppressed, conduction velocity reduced, excitation threshold elevated and both effective refractory period and action potential duration reduced (Opie, 1998; Roden, 1996). The concentration-effect relationships used to model the activities of lidocaine are the E_{max} and sigmoid E_{max}-model (Mazoit *et al.*, 1993).

1.4 Rationale for the study of the pharmacokinetics and pharmacodynamics of sotalol and lidocaine in the presence of inflammatory conditions

Inflammatory diseases have been shown to increase concentrations of AAG, and decrease hepatic clearance, both of which can result in increased drug concentrations (Schneider *et al.*, 1981; Belpaire *et al.*, 1989). Belpaire *et al.*, in 1986 attributed a reduced response to propranolol in rats with inflammation to altered protein binding. Hence, deceased response to propranolol in the inflamed rat was suggested to be due to less free drug interacting with the β-adrenergic receptor. However, several *in vitro* studies show that β-adrenergic function is reduced in the presence of inflammatory mediators (Liu *et al.*, 1999; Chung *et al.*, 1990; Gulick *et al.*, 1989). Therefore, it is not known whether decreased response to drug in laboratory animals with inflammation is due to changes in pharmacokinetics or pharmacodynamics. To address this concern we chose sotalol, β-adrenergic/cardiac potassium blocker, since sotalol is eliminated mainly via the renal route and is negligibly bound to plasma proteins, therefore, inflammation would not be expected to alter its pharmacokinetics. In addition, virtually nothing is known about the activity of cardiac potassium channels in the presence of inflammatory disease. For this purpose, we also chose sotalol to investigate whether cardiac potassium channel response to drug is altered in inflammation in addition to determining whether reduced response to drug is pharmacokinetics dependent.

To date, there is no information that compares the effect of acute and chronic inflammation on the pharmacodynamics of cardiovascular drug. These two types of inflammation may possibly have distinct influences on drug activity due to inherent differences between the two diseases. Therefore, to explore this possibility, sotalol was

administered to rats with acute, $IFN_{\alpha 2a}$-induced, and chronic, *Mycobacterium butyricum*-induced, inflammation.

Cardiovascular drugs that undergo extensive presystemic hepatic metabolism and those highly bound to AAG are candidates for inflammation-induced pharmacokinetic changes. An increased concentration and reduced β-adrenergic and calcium channel activity have been observed in rats with inflammatory disease administered propranolol and verapamil respectively (Guirguis and Jamali, 1996; Mayo *et al.*, 1996). Similar to propranolol and verapamil, lidocaine undergoes extensive first-pass hepatic metabolism, is highly bound to AAG, however, unlike the former two drugs, is metabolized in the rat mainly by CYP2C subfamily and blocks cardiac sodium channels. Therefore, we choose lidocaine to determine whether the effect of inflammation on pharmacokinetics-pharmacodynamics of β-adrenergic and cardiac calcium channel antagonists may be generalized to other cardiovascular drugs that are also highly bound to plasma proteins and undergo extensive first-pass metabolism.

1.5 Hypotheses

1. Acute and chronic inflammation does not alter pharmacokinetics of sotalol enantiomers after administration of racemate and stereoisomers.
2. The pharmacodynamics of sotalol enantiomers are altered in rats with chronic adjuvant arthritis caused by administration of *Mycobacterium butyricum* due to:
    a. Altered responsiveness of β-adrenergic receptors
    b. Altered responsiveness of cardiac potassium channels
3. The pharmacodynamics of sotalol enantiomers are altered in rats with acute $IFN_{\alpha 2a}$-induced inflammation due to:
    c. Altered responsiveness of β-adrenergic receptors
    d. Altered responsiveness of cardiac potassium channels
4. Altered responsiveness to sotalol in inflammation does not differ whether disease is acute, $IFN_{\alpha 2a}$-induced, or chronic, *Mycobacterium butyricum*-induced.
5. The pharmacokinetics of lidocaine does not differ in rats with $IFN_{\alpha 2a}$-induced acute inflammation.
6. The pharmacodynamics of lidocaine does not differ in rats with $IFN_{\alpha 2a}$-induced acute inflammation.

1.6 Objectives

1. To evaluate the effect of adjuvant arthritis on the disposition of sotalol enantiomers after administration of racemate.
2. To evaluate the effect of adjuvant arthritis on responsiveness of β-adrenergic and cardiac potassium channels.
3. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation on the disposition of sotalol enantiomers after administration of racemate.
4. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation on responsiveness of β-adrenergic and cardiac potassium channels after administration of sotalol racemate.
5. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation on the disposition of sotalol enantiomers after administration of stereoisomers.
6. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation on responsiveness of β-adrenergic and cardiac potassium channels after administration of sotalol stereoisomers.
7. To compare the effect of acute and chronic inflammatory disease on β-adrenergic and cardiac potassium channel responsiveness to sotalol.
8. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation *in vivo* on disposition of lidocaine.
9. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation *in vitro* on disposition of lidocaine.
10. To evaluate the effect of $IFN_{\alpha 2a}$-induced inflammation on responsiveness of cardiac sodium channels after administration of lidocaine.

Chapter 2

EXPERIMENTAL

2.1 Chemicals

Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, USA) provided racemate and stereoisomers of sotalol. Atenolol racemate, lidocaine, trimethoprim, verapamil racemate and (+)-glaucine were obtained from Sigma Inc. (Mississauga, ON, Canada). Astra Pharma Inc. (Mississauga, ON, Canada) provided MEGX. $IFN_{\alpha 2a}$ (Roferon A, Hoffmann-La Roche Limited, Mississauga, ON, Canada) was provided by the Cross Cancer Institute of Edmonton.

All reagents required for microsomal incubations and Lowry kits for determination of total protein were purchased from Sigma Inc. (Mississauga, ON, Canada). The derivatizing agent for sotalol assay, S-(+)-1-(1-naphthyl)ethyl isocyanate (NEIC), was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Chloroform, hexane, diethyl ether, isopropyl alcohol, acetonitrile, anhydrous ethanol, n-heptane, ethyl acetate and methanol (Toronto, ON, Canada) were high performance liquid chromatography (HPLC) grade. Acetic acid, phosphoric acid, sulfuric acid, sodium hydroxide, and triethylamine were purchased from BDH (Toronto, ON, Canada). Polyethylene glycol 400 was obtained from Union Carbide (Danbury, CT, USA). Monopotassium phosphate and disodium phosphate required for preparation of Sørensen buffer was bought from BDH (Toronto, ON, Canada). HPLC grade water and squalene were purchased from Fisher Scientific (Edmonton, AB, Canada). Heparin (Heparin Leo 1000 i.u./ml) was purchased from Leo Laboratories (Toronto, ON, Canada). Methoxyflurane (Metofane) was purchased from Janssen Pharmaeutica Inc. (Mississauga, ON, Canada) also killed and dried *Mycobacterium butyricum* (100% w/w) was purchased from Difco Labs (Detroit, MI, USA).

2.2 Assays

2.2.1 Apparatus

The plasma samples were vortexed with a Vortex 2 mixer (Fisher Scientific, Edmonton, AB, Canada) and were centrifuged with a Dynac II centrifuge (Becton Dickinson, Parsippany, NJ, USA). Solvents were evaporated with a Savant Speed Vac concentrator-evaporator (Emerston Instruments, Scarborough, ON, Canada). The HPLC system consisted of a Waters WISP 712 autoinjector and model 590 programmable pump (Waters Inc., Mississauga, ON, Canada). The detectors used were Waters 470 fluorescence detector and Lambda Max 481 UV (Waters Inc.). A Hewlett-Packard model 3390A integrator was used to record the peak areas (Palo, Alto, CA, USA). Diastereomers of sotalol and atenolol (internal standard) were separated on a Phenomenex 25 cm stainless steel 5 μm silica column (Partisil 5, Phenomenex Inc., Mississauga, ON, Canada) using a mobile phase of hexane-chloroform-methanol pumped at 2 ml/min. Lidocaine, MEGX, and trimethoprim (internal standard) were separated on a 10 cm octadecylsilane (ODS) C18 stainless steel 5 μm column (Chromatography Sciences Company Inc., Mississauga, ON, Canada) using a mobile phase of acetonitrile-water-phosphoric acid-triethylamine pumped at 1.3 ml/min. Verapamil enantiomers and (+)-glaucine (internal standard) were separated using a chiral stationary phase composed of 3,5-dimethylphenylcarbamate-derivatized amylose on silica 25 cm stainless steel column (Chiral Pak AD, Diacel Chemical Industries, Tokyo, Japan). Using a mobile phase of hexane-isopropyl alcohol-anhydrous ethanol-triethylamine pumped at a flow

rate of 1 ml/min. Both sample preparation and chromatography were conducted at room temperature.

2.2.2 Standard solutions

Stock solutions of sotalol, atenolol, verapamil, (+)-glaucine and lidocaine were prepared in water to a final concentration of 10 mg/100 ml of the base. MEGX and trimethoprim were prepared in water to a final concentration of 5 mg/25 ml.

The NEIC was diluted with hexane to give a 1 % (v/v) solution and stored under nitrogen at –20° C. To derivatize sotalol and atenolol enantiomers this solution was further diluted with chloroform to give a final concentration of 0.05% (v/v).

2.2.3. Sample preparation and analysis

The following procedures were conducted to determine sotalol, lidocaine and verapamil concentrations in healthy and inflamed rats.

2.2.3.1 Sotalol assay

Plasma sotalol concentrations using 100 µl of rat plasma were measured by a previously reported HPLC method (Carr *et al.*, 1991). Drug free rat plasma was spiked with stock solutions of sotalol racemate to give enantiomer concentrations of 0.05 to 50 µg/ml of each enantiomer. To this was added 50 µl of atenolol (internal standard) and 30 µl of 1 M NaOH. The plasma was vortexed for 30 seconds then centrifuged at 1800 *g* for

5 min with two consecutive 4 ml volumes of ethyl acetate. The two ethyl acetate extractions were combined and evaporated to dryness using a Savant Speed Vac concentrator-evaporator. After drying, the samples were derivatized at room temperature by adding 200 µl of NEIC solution to the residue.

After addition of NEIC the test tubes were vortexed for 30 seconds and aliquots ranging from 75 to 200 µl were injected into the HPLC system and chromatographed using a mobile phase of chloroform-hexane-methanol (65:33:2.0). The fluorescence detector was set at 280 and 335 nm for excitation and emission respectively.

2.2.3.2. Lidocaine assay

Plasma lidocaine and MEGX concentrations using 100 µl of rat plasma were measured by a previously reported HPLC method (Tam *et al.*, 1987). Drug free rat plasma was spiked with stock solutions of to give concentrations of 0.1 to 50 µg/ml of lidocaine and 0.2 to 50 µg/ml of MEGX. To this was added 40 µl of 0.25 M NaOH, 100 µl of trimethoprim (internal standard), and 3 ml of ethyl acetate. The mixture was then vortexed for 30 seconds then centrifuged for 5 min at 1800 *g* then back extracted twice into sulfuric acid pH=2.2 and 150 µl injected into the HPLC system. The mobile phase consisted of water-acetonitrile-phosphoric acid-triethylamine (95:4:0.1:0.5) run at 1.3 ml/min. The ultraviolet detection was at 205 nm.

2.2.3.3. Verapamil assay

Plasma verapamil concentrations using 100 µl of rat plasma were measured by a previously reported HPLC method (Shibukawa and Wainer, 1992). Drug free rat plasma was spiked with stock solutions of verapamil racemate to given enantiomer concentrations of 0.005 to 0.2 µg/ml of each enantiomer. To this was added 75 µl of internal standard (+)-glaucine, 100 µl of 1 M NaOH, 400 µl of Sørensen buffer (pH=7.4) and 6 ml of n-heptane. The plasma was vortexed for 60 seconds, centrifuged at 1800 *g* for 10 min, then evaporated to dryness using a Savant Speed Vac concentrator-evaporator. After drying, the samples were reconstituted in 200 µl mobile phase and 100 µl was injected into the HPLC and chromatographed using a mobile phase of hexane-isopropyl alcohol-anhydrous ethanol-triethylamine (85:7.5:7.5:1.0) run at 1 ml/min. The fluorescence detector was set at 272 and 317 nm for excitation and emission respectively.

2.2.4. Treatment of data

Calibration curves were generated to determine concentrations of sotalol, lidocaine and verapamil. The calibration curves were constructed by plotting peak area ratios of drug to internal standard versus spiked drug concentrations. The calibration curves were evaluated using least squares linear regression analysis. All calibration curves were linear ($r^2 \geq 0.999$) without using a weighting scheme. To evaluate accuracy percent error was calculated from the difference between estimated and added concentrations of drug and was not greater than 20%. The lower limit of quantitation for all the assays used a signal to noise ratio greater than 4:1.

2.3 Pharmacokinetic Studies

Pharmacokinetic studies were conducted to compare disposition of sotalol, lidocaine and verapamil in healthy and inflamed rats.

2.3.1. Induction of adjuvant arthritis

To induce adjuvant arthritis adult male Sprague-Dawley rats weighing (232 ± 6 g) were inoculated in the tail base (intralymphatic) with 175 µl of heat-killed, freeze-dried *Mycobacterium butyricum* suspended in squalene (50 mg/ml). The rats were used approximately 14 days after inoculation. Control rats were not pre-treated.

Before commencement of the experiments, the animals were classified as to the severity of the arthritis using an established method (Whitehouse, 1988). Briefly, an arthritic index (AI) score was assigned by quantifying hind and fore paw swelling by measuring paw diameter using a caliper and by water displacement. In addition, visual assessments were conducted to determine the number of joints affected and presence of arthritic nodules. Each hind paw is visually graded using a score from zero to four with zero representing no swelling or joint involvement, while four includes severe swelling of several joints. Each fore paw is graded from zero to three with zero representing no swelling while a score of three indicates excessive swelling of the wrist and joints. The highest AI score attainable is fourteen indicating severe inflammatory disease with extensive joint involvement.

2.3.2 $IFN_{\alpha 2a}$-induced inflammation

Inflammation was induced in adult male Sprague-Dawley rats (322 ± 34 g) by subcutaneous injections of 5.0×10^4 units of $IFN_{\alpha 2a}$ (Roferon A, Roche Pharmaceuticals, Mississauga, ON, Canada) for two doses at 12 and 3 h prior to administering drug. Rats were inoculated in all experiments at approximately the same time of day in order to avoid potential differences in $IFN_{\alpha 2a}$ priming due to diurnal rhythms of pro-inflammatory cytokines (Petrovsky *et al.*, 1998; Loubaris *et al.*, 1983). To determine affliction with inflammation before commencement of each experiment, a differential bloodstain was performed on each rat. Preparation of the differential stain involved collecting a few drops of blood using a dry syringe via the right jugular vein catheter. Then preparing a blood film by spreading a drop of blood across a standard microscope slide (7.5 × 2.5 cm) approximately 1-1.2 mm thickness (Fischer Scientific, Edmonton, AB, Canada). After the blood film dried then a differential stain (eosine/methylene blue in methanol) was added. After 30 seconds the slide was coated with Sørensen buffer and soaked for 30 min. The buffer was then washed off the slide with distilled water, dried then examined. A total of 100 white cells were counted to determine the percentage of lymphocytes, neutrophils and segmented neutrophils. The amounts of segmented neutrophils (mature neutrophils) were counted since activation of the inflammatory response is thought to accelerate the maturation process (Davies *et al.*, 1999; Dahlgren and Karlsson, 1999). In addition, administration of $IFN_{\alpha 2a}$ is reported to enhance neutrophil respiratory burst a step in which oxidative metabolism of neutrophils increase before phagocytosis which occurs with bacterial and viral infection (Little *et al.*, 1994). Interestingly, oxidative burst responsiveness by neutrophils correlates with severity of inflammation (Hansen *et al.*,

1999). Thus, greater amounts of segmented neutrophils in the $IFN_{\alpha 2a}$ treated rat indicated acute inflammatory disease.

2.3.3. Dosing

Sotalol, lidocaine and verapamil were administered orally. Sotalol racemate, enantiomer and lidocaine were dissolved in normal saline and the appropriate volumes were administered orally using a stainless steel gavage tube. To avoid the influence of biological rhythms on cardiovascular drug disposition and activity (Labreque and Belanger, 1991) rats in all studies were dosed at approximately the same time of day. For the sotalol dose-response study, 10, 20, 40, 80, 100 and 120 mg/kg racemate were administered to healthy adult male Sprague-Dawley rats (n=6/group) and maximum ECG response to drug was determined. For all subsequent sotalol pharmacokinetic-pharmacodynamic studies a dose within the log-linear segment of the dose-response curve of racemate, 40 mg/kg, was administered. Thus, 40 mg/kg of sotalol racemate was administered to healthy and *Mycobacterium butyricum* treated rats (n=4/group). Since sotalol enantiomers are equipotent in potassium channel antagonism but only R-sotalol possesses β-adrenergic blocking activity, single oral doses of 40 mg/kg racemate were tested against 40 mg/kg S- or 20 mg/kg R-sotalol in healthy and $IFN_{\alpha 2a}$ treated rats (n=6/group). We expected equipotent potassium channel and β-adrenergic antagonism following administration of racemate versus S and R enantiomers, respectively. Lidocaine (90 mg/kg), verapamil, (20 mg/kg, positive control) and placebo were administered orally to adult male Sprague-Dawley rats (n=6/group). Verapamil was

dissolved in polyethylene glycol 400 and the appropriate volume was administered by oral gavage.

2.3.4. Surgical procedure and sample collection

In both control and diseased rats, the right jugular vein was catheterized with silastic tubing (0.025 in. i.d. × 0.037 o.d.; Dow Corning, Midland, MI, USA) under a light general anesthesia using methoxyflurane at approximately the same time of day (Janssen Pharmaceuticals, North York, Ontario, Canada). The animals were allowed to recover overnight before the experiment. Rats were allowed access to water, but were fasted the night before the experiment. Single oral doses of racemate and enantiomer were administered to healthy and diseased rats. For all sample collections (0.2 ml/sample) were taken via the right jugular vein catheter. For control and arthritic rats administered sotalol racemate serial blood samples were taken at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h. The control and $IFN_{\alpha 2a}$ treated rats administered sotalol racemate and stereoisomers serial blood samples were taken at 0, 0.25, 0.75, 1.0, 1.5, 4.0 and 6.0 h. Rats administered lidocaine serial blood samples were taken at 0, 0.17, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, and 3.0 h. The rats administered verapamil blood samples were taken at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h. After each collection, the catheter was flushed with an equal volume of heparin in saline (100 i.u./ml). After each blood collection the sample was centrifuged and plasma separated and stored at –20° C until assayed for drug.

2.3.5 Electrocardiograph measurements

For all studies prior to dosing under a light general anesthesia using methoxyflurane three stainless steel Teflon coated electrodes (Cooner wire, Chatsworth, California, USA) were attached to the rats. Two electrodes near the right and left axila regions, and the third at the xiphoid cartilage. To avoid interference of anesthesia with drug and maintain normal body posture rats were conscious during the experiments. Therefore, healthy and inflamed rats were given 3 h to recover from the anesthetic before starting the experiment (Mayo and Jamali, 1999). The electrocardiograph (ECG) measurements PR, RR, and QT intervals were continually monitored using an electronics for medicine Honeywell V1207A Electrocardiograph Amplifier and recorded using Acknowledge III Data Acquisition software (Biopac Systems, Inc., Goleta, California, USA). The PR interval is measured as the distance from the crest of the P wave to the crest of the R wave. The PR interval represents the time required for an impulse to conduct through the tissues located above the ventricles i.e., atria, AV node and His bundle (Goldberger, 2000). The RR interval is measured as the distance from the crest of one R wave to another, and since this represents heart rate will be discussed in the preceding sections as heart rate. In the rat, the ST segment of the electrocardiogram cycle forms a plateau that is not observed in a human ECG. Therefore, in order to quantify the QT interval the distance from the Q dip to the bottom of the ST segment is measured. The QT interval, conduction through Purkinjie fibers and ventricular muscle represents ventricular depolarization and repolarization and may be used to determine cardiac potassium and sodium channel blocking activity (Goldberger, 2000). Prolongation of PR and QT intervals were not corrected for changes in heart rate since a

consistent relationship between the duration of both PR and QT intervals and heart rate in the rat has not been shown (Detweiler, 1981). The net prolongation of the ECG intervals (i.e., PR, RR, and QT), response to drug is reported in milliseconds (msec) or maximum percent change from baseline. Interval measurements were conducted before blood sample collection. For each interval at every sampling time five consecutive cycles were averaged to determine the average interval prolongation at that time.

2.4 In Vitro

A microsome study was conducted to investigate the effect of $IFN_{\alpha 2a}$-induced inflammation on lidocaine metabolism.

2.4.1. Microsomal isolation and incubation

The microsome study was conducted using fresh liver microsomes for both control and inflamed rats. On the day of the experiment, methoxyflurane was used to induce general anesthesia. The portal vein was cannulated and liver perfused with 1.15 percent KCl then removed, weighed, and immersed in ice cold 100 mM sucrose-phosphate buffer (pH=7.4). The liver was then minced, homogenized, and centrifuged at 10,000 *g* for 20 min. The supernatant was collected and centrifuged at 105,000 *g* for 60 min; the resultant protein pellet was resuspended and again centrifuged at 105,000 *g* for 60 min. The final pellet was suspended in sucrose-phosphate buffer (pH=7.4). Protein content was determined using the Lowry method (Lowry *et al.*, 1951). The incubation

mixture was a total volume of 3 mls and consisted of: 5 mM $MgCl_2$, 10 mM Glucose-6-phosphate, 0.75 mM $NADP^+$, 2 U/ml glucose-6-phosphate dehydrogenase, and 2 mg/ml of liver microsomes in 0.05 M Tris-HCL buffer (pH=7.4). The mixture was spiked with 2.5 μg/ml lidocaine and gently shaken in a water bath at 37° C for 30 min. To stop the reaction at 0, 5, 10, 15, 20, 25, 30 min after starting the incubation 0.2 ml of incubation mixture was transferred to test tubes containing 0.2 ml of 1 M NaOH. The samples were stored at -20° C until assayed for lidocaine and MEGX. Microsomal metabolism by control livers were compared to that of test by measuring lidocaine concentrations and plotting cumulative percent metabolism of lidocaine versus incubation time. Formation of MEGX by control and inflamed rat livers was determined by measuring MEGX concentrations and plotting cumulative percent formation of MEGX with 30 minutes considered to be 100 % formation of metabolite versus incubation time.

2.5 Data Analysis

2.5.1. Pharmacokinetic indices

Pharmacokinetic parameters were determined by WinNonlin standard version 1.0 (Scientific Consulting Inc., Apex, NJ, USA). Noncompartmental methods were used to describe the disposition of sotalol, lidocaine, and verapamil in which assumption of a specific compartmental model is not required (Gillespie, 1991). Plasma concentrations of sotalol enantiomers, lidocaine and MEGX for control and inflamed rats are compared on concentration versus time plots in which the concentration scale is logarithmic. The AUC

is calculated using the trapezoidal rule. The $AUC_{0-\infty}$ is determined from C_{Last}/β where C_{Last} is the last point on the concentration-time curve and β is the terminal elimination rate constant, which was calculated using at least three points in the log-linear terminal phase of the plasma concentration-time curve. The oral clearance (CL/F) is calculated using $Dose/AUC_{0-\infty}$ where F is the oral bioavailability. The oral volume of distribution (Vd/F) is estimated from $Dose/AUC_{0-\infty}*\beta$. To determine the *in vitro* correlation between lidocaine metabolism, formation of MEGX, and incubation time, cumulative percent metabolism of lidocaine and formation of MEGX were fitted to a Hill equation using SigmaPlot for Windows version 4.0 (SPSS Inc., Chicago, IL, USA).

2.5.2 Pharmacodynamic indices

To determine the correlation between dose of sotalol and ECG interval prolongation for the dose-response study the effect of escalating doses of sotalol racemate on PR and QT interval prolongation were fitted to a Hill equation using SigmaPlot for Windows version 4.0 (SPSS Inc., Chicago, IL, USA). The area under the effect curve of interval prolongation during the sampling period was truncated from 0 to 6 h ($AUEC_{0-6h}$) for sotalol and 0 to 3 h ($AUEC_{0-3h}$) for lidocaine and calculated using the trapezoidal rule.

2.5.3. Statistical comparisons

Statistical significance was evaluated using a Student's *t*-test by comparing control to inflamed rats. Non-linear regression analysis was used to determine the adequacy of curve fitting for the dose response curves and microsomal study. Least squares regression analysis and Pearson's correlation coefficient was used to assess the significance of these relationships. All tests were conducted with a level of confidence set at α=0.05. The data are reported as mean ± standard deviation.

Chapter 3

REDUCED β-ADRENERGIC AND POTASSIUM CHANNEL ANTAGONIST ACTIVITIES OF SOTALOL IN RATS WITH ACUTE AND CHRONIC INFLAMMATORY CONDITIONS

3.1 Results

At approximately 10-14 days after intralymphatic inoculation with *Mycobacterium butyricum*, skin nodules on the ears and tail, as well as fore and hind paw swelling were noted. The AI scores ranged from 10-12 indicating moderate adjuvant arthritis. Treatment with $IFN_{\alpha 2a}$ resulted in significantly greater counts of segmented neutrophils as compared with control rats (Table 3-1).

From plots of racemic sotalol doses versus prolongation of PR and QT intervals in healthy rats the 40 mg/kg dose was chosen for pharmacokinetic-pharmacodynamic studies since it produced effects in the ascending phase of the dose-response curves (Figure 3-1).

To determine the effect of inflammation on ECG response to sotalol both individual data points (Figure 3-2 and 3-3) and $AUEC_{0\text{-}6h}$ (Table 3-2 and 3-3) were statistically analyzed. No significant differences in baseline PR and QT intervals were found between the control and inflamed rats (Figure 3-2 and 3-3). These baseline values were close to those previously reported (Detweiler *et al.*, 1981). With regard to heart rate, however, despite similar baseline values in healthy and $IFN_{\alpha 2a}$-treated rats, chronic arthritis caused a significantly greater baseline value (Figure 3-2). Sotalol reduced heart rate in both groups of rats. The rate returned to baseline values in 6 h (Figure 3-2 and 3-3). No significant differences in the effect of sotalol on heart rate was noticed between controls and either rats with adjuvant arthritis (Figure 3-2) or the acutely inflamed animals (Figure 3-3). On the other hand, the influence of sotalol on PR and QT intervals was significantly less pronounced in both chronic (Figure 3-2 and Table 3-2) and acutely inflamed (Figure 3-3 and Table 3-3) rats as compared with controls. As expected, while

both racemic and R-sotalol prolonged PR and QT intervals and S-sotalol lengthened only QT interval (Figure 3-3 and Table 3-3).

Plots of effect versus sotalol concentration exhibited high degrees of variability. Nevertheless, PR and QT interval-concentration relationships were in general sigmoidal in shape on the other hand heart rate concentration curves were better described as a counter-clockwise hysteresis (Figure 3-4). Attempts to collapse the latter failed due, perhaps, to excessive variability. No further interpretation of pharmacokinetic-pharmacodynamic data was made due to variability.

As anticipated chronic and acute inflammation did not influence pharmacokinetics of sotalol enantiomers after administration of racemate and stereoisomers (Figure 3-5 and 3-6, Tables 3-4 and 3-5). After administration of sotalol racemate to healthy and inflamed rats the AUC of both R and S-enantiomers were not different thus disposition was not stereoselective (Tables 3-4 and 3-5).

3.2 Discussion

The present data suggest reduced sotalol potency to prolong PR and QT intervals in rats with inflammation. This may be due to an altered pharmacokinetics or a reduced receptor responsiveness. The former explanation can be ruled out since the pharmacokinetics of sotalol remained unaffected by inflammation (Figure 3-5 and 3-6, Tables 3-3 and 3-4). The observed lack of an inflammation-induced pharmacokinetics alteration was expected. Sotalol is negligibly plasma protein bound (Belpaire *et al.*, 1982) and is almost completely cleared via the renal pathway (Anderson and Prystowsky,

1999). Hence, the two major inflammation-induced changes i.e., increased serum AAG and decreased intrinsic hepatic clearance (Piafsky *et al.*, 1978; Belpaire *et al.*, 1989) do not play any significant role in clearance of the drug. This suggests the decreased response to the cardiovascular drug in the presence of inflammation is due to a pharmacodynamic rather than a pharmacokinetic disease-drug interaction.

A series of published *in vitro* data may help explain our *in vivo* observation that suggests reduced potency of sotalol. Exposure of isolated cardiac myocytes to pro-inflammatory cytokines such as IL-1β (Gulick *et al.*, 1989; Lui *et al.*, 1999;) and TNFα has been shown to decrease β-adrenergic responsiveness. Nitric oxide produced by ventricular myocytes has been suggested to contribute to the IL-1β-induced reduction in β-adrenergic activity (Rozanski and Witt, 1994). However, decreased β-adrenergic responsiveness has also been observed independent of increased nitric oxide production (Muller-Werdan *et al.*, 1998). Therefore, the observed reduced potency of sotalol to prolong PR interval in inflamed rats is due, perhaps, to an altered cardiovascular receptor function causing an alteration in β-adrenergic receptor configuration, density and/or chemical messenger activity secondary to an over-expression of pro-inflammatory cytokines.

The higher baseline heart rate observed in the adjuvant rat is perhaps due to higher levels of catecholamines that may be a contributing factor to the decreased PR interval prolongation after administration of sotalol racemate. Noradrenaline as a relatively selective β_1-adrenergic receptor agonist downregulates β_1-adrenergic receptors in rats with pheochromocytoma by altering receptor function and density (Tsujimoto *et al.*, 1984). Consequently, the enhanced sympathetic tone in the adjuvant rat may

contribute to the reduced responsiveness of the PR interval to sotalol. However, after administration of sotalol racemate, rats with acute inflammation exhibited a similar reduction in PR interval prolongation as that observed in chronic arthritic animals. Therefore, the enhanced sympathetic tone in the rat with chronic arthritis did not significantly contribute to the reduced PR interval responsiveness to sotalol racemate.

Beta-adrenergic antagonists have been found to be less efficacious in treating hypertension in the elderly as compared with younger individuals (Brodde *et al.*, 1995; Messerli *et al.*, 1998; Tenero *et al.*, 1990). Interestingly, it has been shown that there are increased levels of certain pro-inflammatory cytokines [i.e., TNFα, IL-1, IL-6] in the elderly (Bruunsgaard *et al.*, 1999; Liao *et al.*, 1993). In addition, older individuals taking β-blocker atenolol do not appear to experience benefit of reduction in cardiovascular disease or mortality (MRC Working party, 1992). It appears timely to investigate whether these observations are linked to altered cytokine expression as we have reported in our animal models. Clinically, therefore, altered responsiveness to β-adrenergic antagonists may occur in various circumstances where increased concentrations of pro-inflammatory cytokines are present such as individuals having inflammatory conditions and in the elderly.

Our data also suggest a down-regulation of potassium channel receptors in addition to that of β-adrenergic receptors in both chronic and acute inflammations. Racemic sotalol antagonizes both these receptors (Anderson and Prystowsky, 1999). Hence, both PR and QT intervals are prolonged and inflammation seems to reduce the effect of sotalol on both of these ECG intervals. Nevertheless, to confirm the effect and to unequivocally demonstrate reduced response of potassium channel receptors in

inflammation, we took advantage of stereochemistry of sotalol. The effect of S-sotalol, the specific potassium channel blocker enantiomer (Anderson and Prystowsky, 1999), on QT intervals was also reduced by inflammation. It should be mentioned that both racemic and R-sotalol also affected QT interval but, as expected, this was accompanied by a similar effect on PR interval due to their lack of specificity. In support of our finding, recently it has been shown that administration of $IFN_{\alpha 2a}$ to rabbits ventricular myocytes reduces *in vitro* ATP-sensitive potassium channel activity (Nishio *et al.*, 1999). A potassium channel down-regulation has also been observed *in vitro* using ventricular myocytes from rats that were subjected to an experimentally-induced myocardial infarction (Kaprielian *et al.*, 1999). Interestingly, pro-inflammatory cytokines have been reported to be elevated after myocardial infarction (Neumann *et* al., 1995) this is coupled with a reduced potassium channel activity in the myocytes a relationship between these two factors, although unknown, is plausible. Similarly, overexpression of pro-inflammatory cytokines and potassium channel down-regulation is observed in patients with congestive heart failure (Seta *et al.*, 1996; Nabauer and Kaab, 1998). This increased expression of inflammatory mediators [i.e., TNFα and IL-6] may possibly decrease potassium channel activity which, in turn, results in the electrical instability and abnormal ventricular repolarization observed in heart failure (Nabauer and Kaab, 1998). Hence, the decreased QT interval prolongation in sotalol-treated rats with acute and chronic arthritis is also due, perhaps, to increased expression of pro-inflammatory cytokines.

In addition to β-adrenergic and potassium channel receptors cardiac calcium channel responsiveness appears to be affected by increased expression of pro-inflammatory cytokines (Mayo *et al.*, 1996). Interestingly, Abernethy et al have shown a

decreased sensitivity of calcium channels to verapamil in older individuals (Abernethy *et al.*, 1993) and obese hypertensive patients (Abernethy and Schwartz, 1988). Increased expression of pro-inflammatory cytokines reported for elderly (Bruunsgaard *et al.*, 1999; Liao *et al.*, 1993) and obese subjects (Visser *et al.*, 1999) may also explain the reduction in calcium channel sensitivity.

The decreased β-adrenergic receptor and cardiac potassium channel function was observed in both acute and chronic inflammation. Acute inflammation is characterized by sudden onset, short duration and is accompanied by production of acute phase proteins by the liver (Kuby, 1997). Acute inflammatory disease was induced by inoculating rats with $IFN_{\alpha 2a}$ which stimulates Th1 cells to secrete pro-inflammatory cytokines IFNγ and IL-2 (Tilg and Peschel, 1996). Administration of the cytokine caused acute inflammation since rats inoculated with $IFN_{\alpha 2a}$ developed an inflammatory response within 24 h which was confirmed by an increased segmented neutrophils count compared to control rats. In addition, the inflammation subsided within 1 week thus is a model for acute inflammation. Unlike $IFN_{\alpha 2a}$-induced inflammation, chronic inflammatory diseases such as that induced by injection of *Mycobacterium butyricum* are associated with a persistent antigen that resists phagocytosis (Kuby, 1997). The bacterium was suspended in squalene which prolongs the exposure of this antigen to the rat since it is released slowly and the squalene hinders phagocytosis of the *Mycobacterium* (Whitehouse *et al.*, 1974). Thus, a chronic inflammatory response that took 14 days to develop was observed in the bacterium treated rat. Inflammation caused by injection of the adjuvant resulted in greater inflammatory responses and tissue damage than observed after $IFN_{\alpha 2a}$ inoculation. Inflammatory cytokines e.g., IFNγ and TNFα, contribute to the development and

progression of chronic inflammatory diseases, which, in turn, results in activation of macrophages and subsequent tissue damage (Kuby, 1997). Interestingly, sotalol pharmacodynamics was affected by both chronic and acute inflammation despite the inherent differences between these two types of inflammation.

The reduced potency of sotalol in rats inoculated with $IFN_{\alpha 2a}$ may also be considered a drug interaction since $IFN_{\alpha 2a}$ and other cytokines are used to treat various diseases. The clinical significance of our observation remains to be explored in patients with conditions such as chronic active hepatitis B and renal cell carcinoma who are recipients of $IFN_{\alpha 2a}$ therapy. Interestingly, in these disorders, development of arthritis as a complication of $IFN_{\alpha 2a}$ treatment, has been reported (Nesher and Ruchlemer, 1998). Therefore, $IFN_{\alpha 2a}$ has an important role in both the pathogenesis and treatment of disease and the altered sensitivity of cardiovascular receptors to sotalol observed in this study may be extrapolated to a drug-drug interaction in addition to drug-disease interaction.

The altered response to sotalol in the rat with both acute and chronic inflammation emphasizes the importance of disease-drug interactions. As with most drugs there are discrete sub-populations (e.g., disease, age, concurrent therapy) for whom concentration-effect relationships differ from what is commonly seen and understood (Levy, 1998). Interestingly, inflammatory status has been shown to determine the clinical course of post-myocardial infarction patients who have unstable angina and receive standard drug therapy (Verheggen et al., 1999). In addition, mortality in post-myocardial infarction was greater in individuals with higher concentrations of inflammatory marker, CRP, independent of drug treatment (Pietila *et al.*, 1996). Therefore, our observation in the rat may shed light into the variability in response to cardiovascular agents in the

treatment of hypertension and arrhythmia in patients with elevated pro-inflammatory cytokine concentrations due to a variety of pathophysiological changes.

3.3 Conclusions

Inflammation caused by administration of *Mycobacterium butyricum* and $IFN_{\alpha 2a}$ did not alter pharmacokinetics of sotalol after administration of racemate and stereoisomers. This indicates that reduced response to cardiovascular drug in inflammation is not pharmacokinetics dependent. Both acute and chronic inflammation reduced the effect of R-sotalol and racemate on PR interval and decreased the potency of stereoisomers and racemate on QT interval. Therefore, acute and chronic inflammations altered β-adrenergic and cardiac potassium channel function. The reduced β-adrenergic and cardiac potassium channel activity in the inflamed rat despite no changes in enantiomer concentrations indicates that pharmacokinetics may not always reflect pharmacodynamics.

Table 3-1: Mean ± standard deviation of hematological parameters after second injection of $IFN_{\alpha 2a}$ 5.0×10^4 units subcutaneous 3 h before oral dosing of racemate (40 mg/kg), R (20 mg/kg) and S (40 mg/kg) sotalol. Control and inflamed rats (n=6/group), *denotes significantly different from control ($p<0.05$).

	Lymphocytes (%)	Neutrophils (%)	Segmented Neutrophils (%)
Control			
Racemate	80 ± 4	14 ± 3	5 ± 2
R-sotalol	80 ± 6	15 ± 4	4 ± 3
S-sotalol	79 ± 5	16 ± 4	6 ± 2
$IFN_{\alpha 2a}$-Treated			
Racemate	77 ± 2	8 ± 2*	16 ± 2*
R-sotalol	76 ± 3	7 ± 2*	17 ± 4*
S-sotalol	80 ± 4	5 ± 1*	15 ± 4*

Table 3-2: Truncated area under the effect curves from 0 to 6 h following oral administration of racemate (40 mg/kg) to control and chronic adjuvant arthritic rats (n=4/group). Values as expressed as mean ± standard deviation, *denotes significantly different ($p<0.05$).

	PR Interval (msec×h)		QT Interval (msec×h)		Heart Rate (msec×h)	
	Control	Arthritic	Control	Arthritic	Control	Arthritic
Racemate	264 ± 5	248 ± 3*	414 ± 10	388 ± 9*	1968 ± 90	2051 ± 139

Table 3-3: Truncated area under the effect curves from 0 to 6 h following oral administration of racemate (40 mg/kg), S (40 mg/kg) and R (20 mg/kg) sotalol to control and rats treated with $IFN_{\alpha 2a}$ (n=6/group). Values as expressed as mean ± standard deviation, *denotes significantly different ($p<0.05$).

	PR Interval (msec×h)		QT Interval (msec×h)		Heart Rate (msec×h)	
	Control	Inflamed	Control	Inflamed	Control	Inflamed
Racemate	262 ± 5	249 ± 9*	416 ± 7	375 ± 8*	1924 ± 115	1941 ± 116
S-sotalol	242 ± 4	247 ± 6	409 ± 12	373± 18*	1982 ± 154	1968 ± 59
R-sotalol	255 ± 5	241 ± 4*	390 ± 16	371 ± 5*	1885 ± 115	1976 ± 65

Table 3-4: Mean ± standard deviation of pharmacokinetic indices following administration of 40 mg/kg racemic sotalol to healthy and chronic arthritic rats, no significant differences were found between the two groups ($p>0.05$).

	Control 40 mg/kg		Arthritic 40 mg/kg	
	S	R	S	R
T_{max} (h)[a]	2.00 ± 0.00	2.00 ± 0.00	1.56 ± 0.59	1.56 ± 0.59
C_{max} (µg/ml)[b]	4.78 ± 0.96	4.76 ± 0.94	5.29 ± 1.89	5.40 ± 1.62
$T_{1/2}$ (h)[c]	1.43 ± 0.41	1.47 ± 0.43	1.40 ± 0.26	1.37 ± 0.22
AUC_{0-6} (µg*h/ml)[d]	14.65 ± 1.87	14.15 ± 1.58	12.40 ± 3.50	12.50 ± 2.85
$AUC_{0-\infty}$ (µg*h/ml)[e]	16.27 ± 1.35	15.74 ± 1.44	13.20 ± 3.83	13.40 ± 3.03
CL/F (L/h)[f]	0.38 ± 0.03	0.40 ± 0.03	0.43 ± 0.12	0.42 ± 0.10
Vd/F (L)[g]	0.80 ± 0.28	0.84 ± 0.26	0.88 ± 0.40	0.83 ± 0.33

[a] Time at maximum observable plasma concentration

[b] Maximum observable plasma concentration

[c] Terminal elimination half-life estimated from at least 3 data points of each rat

[d] Truncated area under the plasma concentration-time curve from 0 to 6 h

[e] Area under the plasma concentration-time curve extrapolated to infinity

[f] Oral clearance

[g] Oral volume of distribution

Table 3-5: Pharmacokinetic indices following administration of racemate (40 mg/kg), S (40 mg/kg) and R (20 mg/kg) sotalol to control and $IFN_{\alpha 2a}$ treated rats (n=6/group). Values are expressed as mean ± standard deviation, no significant differences were found between control and inflamed rats ($p>0.05$).

	T_{max} (h)[a]		C_{max} (ug/ml)[b]		AUC_{0-6} (ug*h/ml)[c]	
	Control	$IFN_{\alpha 2a}$	Control	$IFN_{\alpha 2a}$	Control	$IFN_{\alpha 2a}$
Racemate						
S-sotalol	1.38 ± 0.31	1.54 ± 1.26	4.15 ± 0.76	3.61 ± 1.54	14.22 ± 2.66	12.99 ± 2.95
R-sotalol	1.38 ± 0.31	1.54 ± 1.26	4.23 ± 0.84	3.91 ± 2.02	14.16 ± 2.84	13.87 ± 3.42
Enantiomer						
S-sotalol	1.54 ± 1.26	1.38 ± 0.31	7.91± 2.46	8.77± 3.44	29.14 ± 5.03	27.65 ± 8.44
R-sotalol	1.79 ± 1.12	1.54 ± 1.26	3.42 ± 1.19	3.80 ± 2.37	13.45 ± 3.69	14.00 ± 7.93

[a] Time at maximum observable concentration

[b] Maximum observable concentration

[c] Truncated area under the plasma concentration-time curve from 0 to 6 h

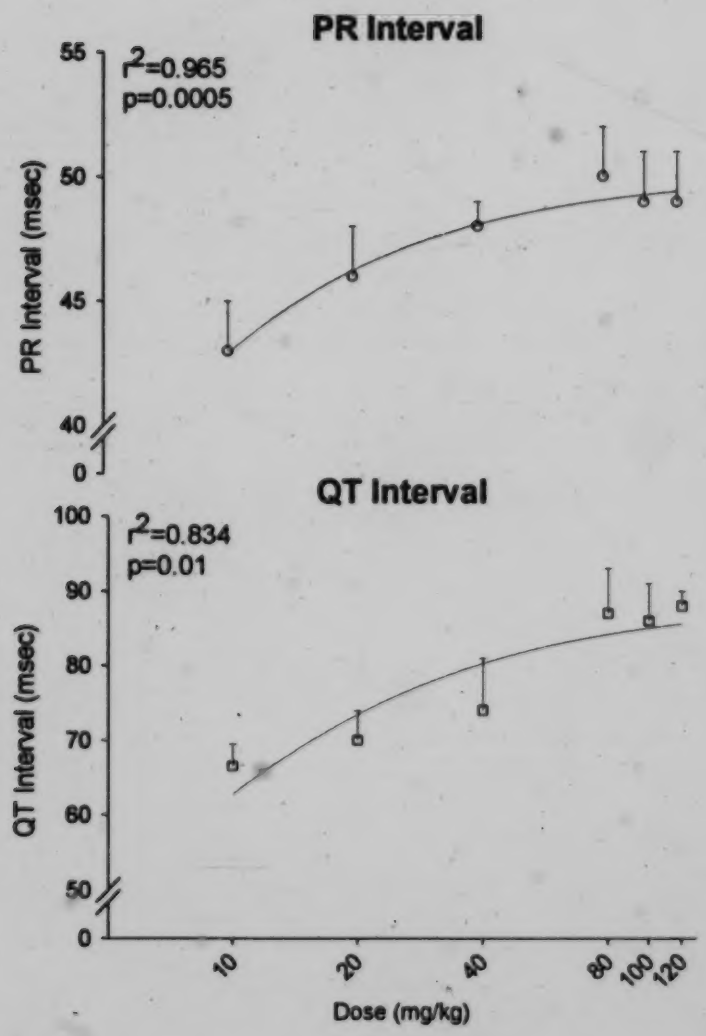

Figure. 3-1: Maximum prolongation of PR [KK1](O) and QT (□) intervals after administration of various doses of sotalol racemate. Error bars represent standard deviation of the mean, lines through the data points represent the best fit using hyperbola function.

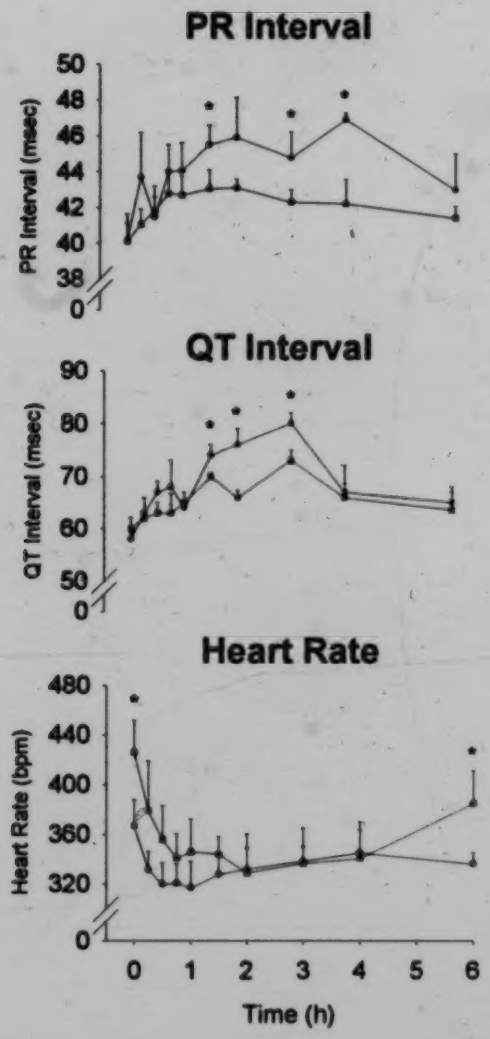

Figure. 3-2: Time courses of sotalol effect following administration of single oral doses of racemic sotalol (40 mg/kg) to healthy and arthritic rats. Control (O) and arthritic (□), error bars represent standard deviation of the mean, *denotes significantly different ($p<0.05$).

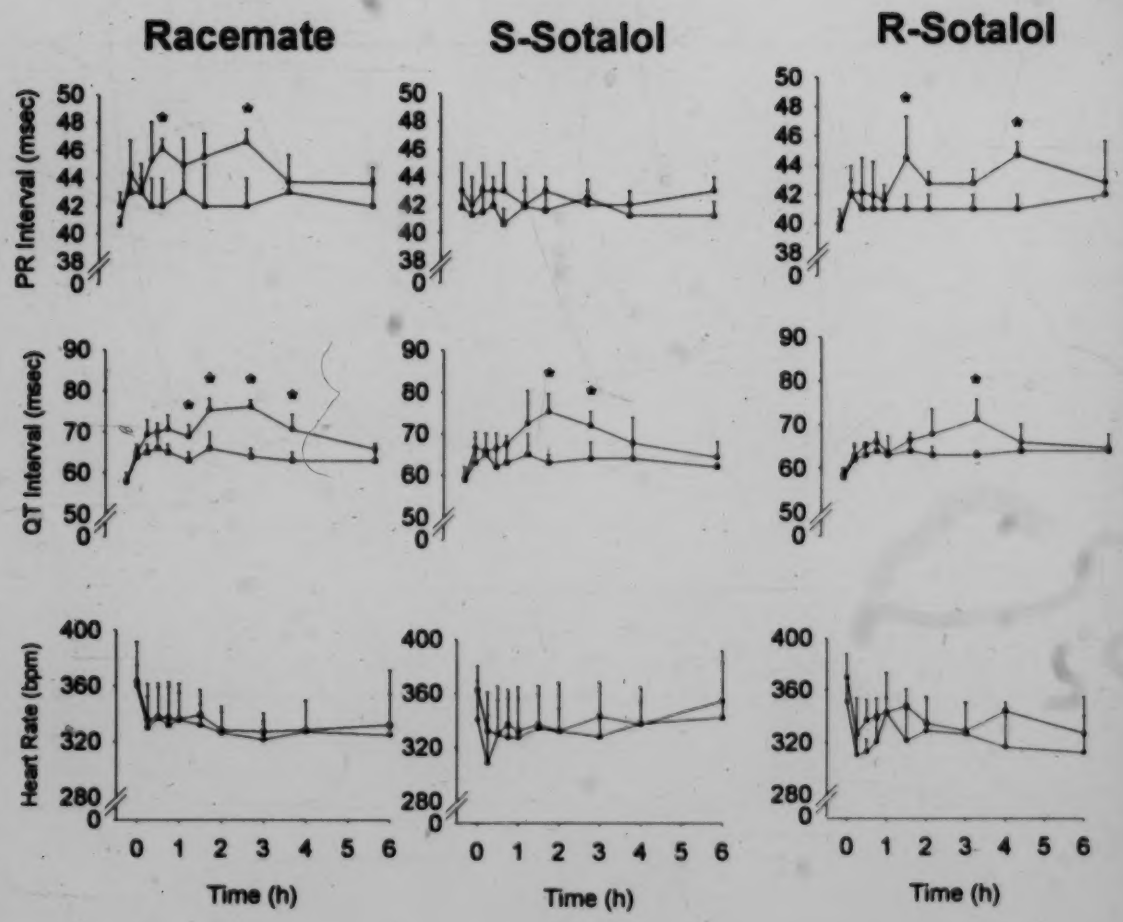

Figure. 3-3: Time courses of sotalol effect following administration of single oral doses of 40 mg/kg sotalol racemate, S-sotalol (40 mg/kg) and R-sotalol (20 mg/kg) to control and $IFN_{\alpha 2a}$ treated rats. Control (O) and inflamed (□), error bars represent standard deviation of the mean, * denotes significantly different ($p<0.05$).

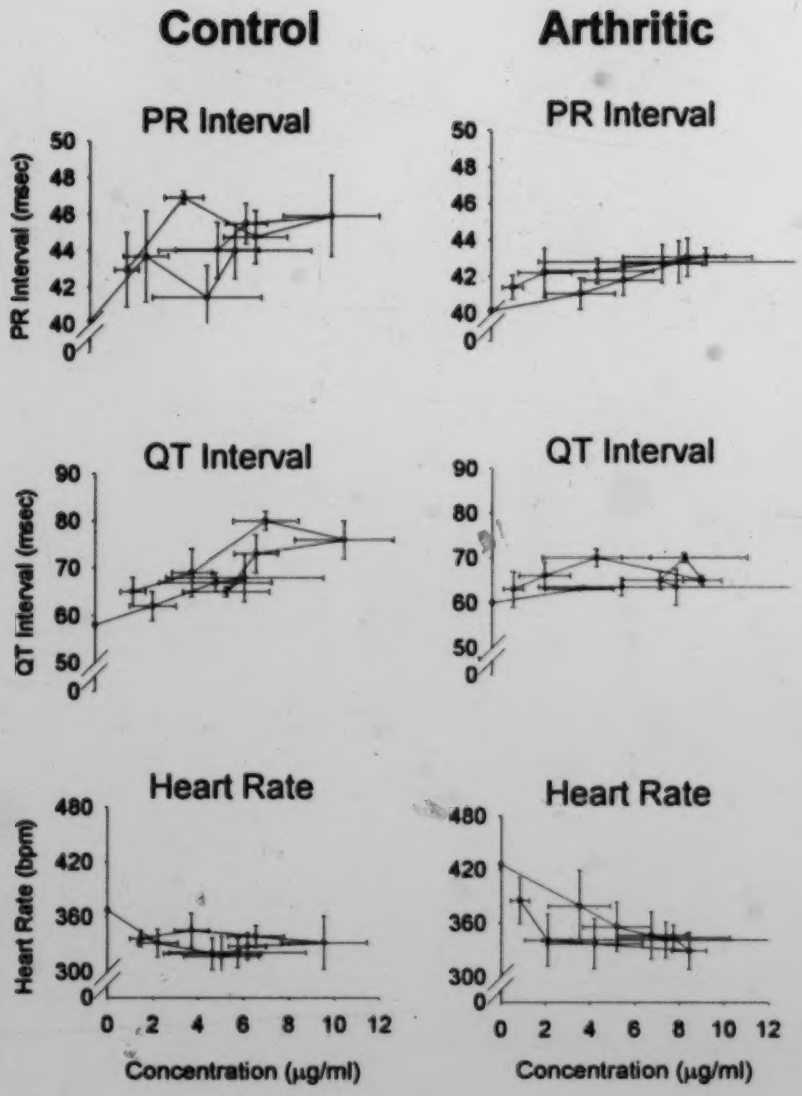

Figure 3-4: ECG interval versus plasma concentration of sotalol after administration of racemate (40 mg/kg) to control and chronic arthritic rats. The data points are connected in temporal order commencing from the baseline interval at time zero.

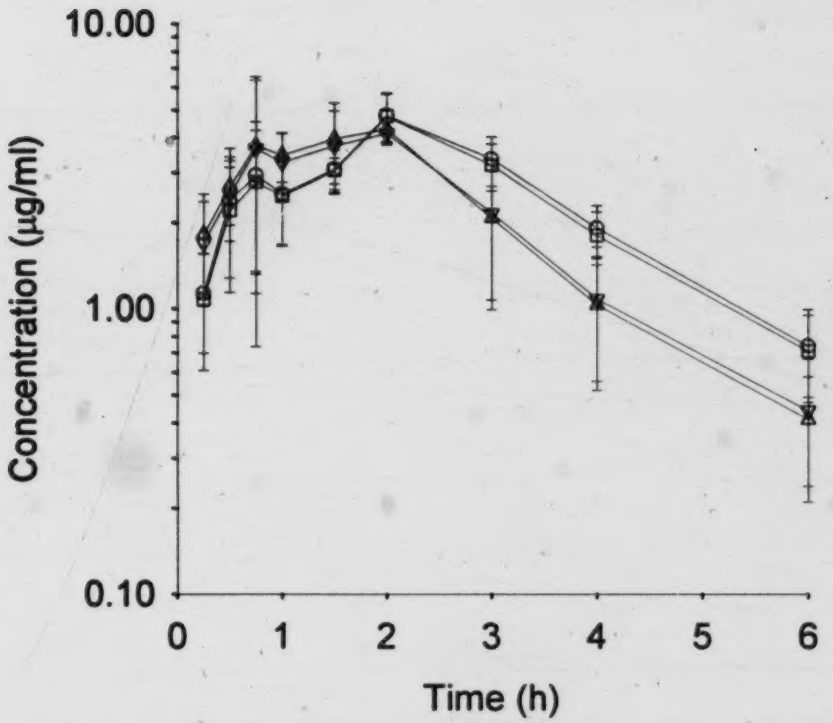

Figure. 3-5: Plasma sotalol enantiomer concentration-time plots in control and rats with chronic arthritis following racemate (40mg/kg) oral doses. Control R (□), and S (O), arthritic R (Δ) and S (∇), error bars represent standard deviation of the mean no significant differences between control and inflamed rats are observed ($p>0.05$).

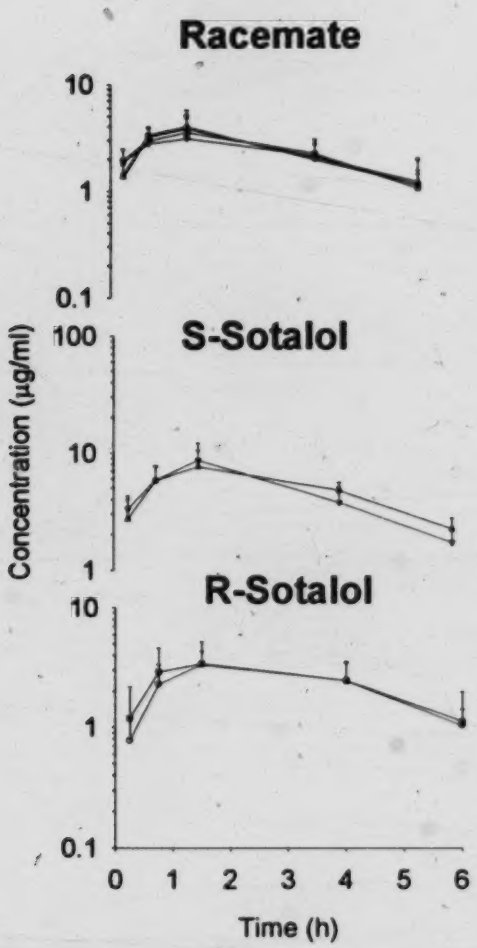

Figure. 3-6: Plasma sotalol enantiomer concentration-time plots in control and $IFN_{\alpha 2a}$ treated rats following racemate (40mg/kg), S-sotalol (40 mg/kg) and R-sotalol (20 mg/kg) oral doses. Control R (O), and S (Δ), $IFN_{\alpha 2a}$ treated R (□) and S (∇), error bars represent standard deviation of the mean no significant differences between control and inflamed rats are observed ($p>0.05$).

Chapter 4

DISPOSITION OF LIDOCAINE AND CARDIAC SODIUM CHANNEL RESPONSIVENESS IS NOT INFLUENCED BY INTERFERON$_{\alpha 2a}$-INDUCED ACUTE INFLAMMATION

4.1 Results

Rats inoculated with $IFN_{\alpha 2a}$ had significantly greater amounts of segmented neutrophils compared to controls (Table 4-1). Figure 4-1 depicts stereoselective metabolism of verapamil enantiomers and reduction of oral clearance of R and S enantiomers in the inflamed rat. As shown in Figure 4-2, while no differences in heart rate are observed between control and test rats administered verapamil, significantly less prolongation of the PR interval is seen in the rat with inflammation.

$IFN_{\alpha 2a}$-induced inflammation did not alter pharmacokinetics of lidocaine or MEGX (Figure 4-3 and Table 4-2). Pharmacokinetic parameters of lidocaine are close to those previously reported from a dose-dependency study which demonstrated linear pharmacokinetics for the 50 to 90 mg/kg dose range (Supradist *et al.*, 1984).

To determine the effect of acute inflammation caused by $IFN_{\alpha 2a}$ treatment on ECG response to lidocaine both individual data points (Figure 4-4) and $AUEC_{0\text{-}3h}$ (Table 4-3) were statistically analyzed. No significant differences in baseline PR, RR (heart rate) and QT intervals were found between healthy and inflamed rats (Figure 4-4) and baseline values are similar to previously reported values (Detweiler, 1981). No significant differences in PR and QT intervals and heart rate responses to lidocaine were observed between healthy and inflamed rats (Figure 4-4). The $AUEC_{0\text{-}3h}$ of PR interval prolongation was not different comparing healthy to inflamed rats (Figure 4-4, Table 4-3). Similar to PR interval $AUEC_{0\text{-}3h}$ of QT interval and heart rate reduction after administration of lidocaine was not altered by the cytokine-induced inflammation (Figure 4-4, Table 4-3).

Plots of ECG interval responses versus lidocaine concentration exhibited high variability. For both PR and QT intervals, a sigmoidal relationship between interval response and lidocaine concentration was observed. A hysteresis loop with counter-clockwise rotation for heart rate reduction versus lidocaine concentration was also observed, however, attempts to collapse the hysteresis failed due to high variability. Due to the variability, no further attempts at pharmacokinetic-pharmacodynamic modeling were conducted.

Microsomal oxidative activity was evident for both control and $IFN_{\alpha 2a}$ treated rats. The cumulative percent metabolism of lidocaine and percents of total MEGX formed over 30 min of incubation was not affected by acute inflammatory disease (Figure 4-5).

4.2 Discussion

Lidocaine, propranolol and verapamil are candidates for inflammation-induced pharmacokinetic changes since these drugs are highly bound to AAG and undergo extensive presystemic hepatic metabolism. However, unlike propranolol and verapamil, lidocaine is mainly metabolized in the rat by CYP2C isozymes and blocks fast sodium channels in Purkinjie fibers and ventricular muscle (McEvoy, 1999). Therefore, to determine whether acute inflammation caused by administration of $IFN_{\alpha 2a}$ influences CYP2C subfamily and cardiac sodium channel activities lidocaine was administered to healthy and acutely inflamed rats.

Oral bioavalibility of lidocaine is low due to high first-pass metabolism, therefore to treat and prevent ventricular arrhythmia associated with myocardial infarction, surgery and ventricular tachycardia lidocaine is administered intravenously (Pieper and Johnson, 1992). Despite intravenous administration of lidocaine to patients with cardiovascular disease, by giving lidocaine orally to healthy and inflamed rats, one can use lidocaine as a probe to investigate the effect of acute inflammation on CYP isozyme function. Oral administration is essential to evaluate the effect of inflammation on drugs that have a high first-pass metabolism (Belpaire *et al.*, 1989). Since decreases in intrinsic hepatic clearance of highly metabolized drugs, generally result in decreased presystemic rather than systemic clearance (Gibaldi and Perrier, 1982). For example, after intravenous administration of propranolol disposition of drug was similar in healthy and inflamed rats, however, after oral administration inflamed rats had significantly greater concentrations of propranolol enantiomers compared to controls (Piquette-Miller and Jamali, 1993). In addition, inflammation reduced presystemic but not systemic clearance of metoprolol a drug that also undergoes extensive first-pass hepatic metabolism (Belpaire *et al.*, 1989). Therefore, lidocaine was administered orally to assess the effect of acute inflammation produced by $IFN_{\alpha 2a}$ inoculation on hepatic isozyme function. The major metabolite of lidocaine MEGX was quantitated in order to further examine the affect of inflammation on isozyme function. In addition, MEGX possess approximately 83% of the antiarrhythmic activity of parent drug thus formation of this metabolite significantly contributes to ECG responses after administration of parent drug (Rodighiero, 1989).

It is reported that disposition of lidocaine is not altered by $IFN_{\alpha 2a}$ treatment (Melzer *et al.*, 1994). However, since lidocaine was administered via the intravenous route, therefore, the effect of acute inflammation on isozyme function could not be determined. However, suspecting that acute inflammation may not alter lidocaine metabolism, verapamil was used as a positive control as previously $IFN_{\alpha 2a}$-induced inflammation was shown to reduce oral clearance of verapamil and sensitivity to drug (Mayo *et al.*, 1996). With this in mind, since lidocaine pharmacokinetics is reported to not be altered by $IFN_{\alpha 2a}$-induced acute inflammatory disease receptor response to drug i.e., cardiac sodium channel may be determined in the absence of complications of altered pharmacokinetics.

In rats N-dealkylation of lidocaine to MEGX is catalyzed mainly by CYP2C subfamily [i.e., CYP2C11] with CYP1A2, 2B1, 3A2 and 2D1/2 having less significant roles (Masubuchi *et al.*, 1991; 1993; Oda *et al.*, 1989). Therefore, to determine the effect of $IFN_{\alpha 2a}$-induced inflammation on CYP2C activity concentrations of both lidocaine and MEGX in control and inflamed rats were compared. There also exists a gender difference in formation rates of lidocaine metabolites (Lennard *et al.*, 1983) arising from preferential expression of CYP2C11 and CYP3A2 in the male rat, but not in the female rat. Hence, male rats were used in this study (Smith, 1991). Concentrations of major metabolite, MEGX, were also measured since formation kinetics of MEGX is reported to be sensitive to hepatic isozyme function (Esen *et al.*, 1997; Pieper and Johnson, 1992). Interestingly, formation of MEGX after lidocaine administration has been used to assess metabolic impairment in severe sepsis, liver function, and enzyme activity before and after liver transplantation (Esen *et al.*, 1997; Reichel *et al.*, 1997).

Despite a reduced oral clearance of verapamil enantiomers in the rat with inflammation caused by administration of the cytokine similar concentration-time curves of lidocaine, MEGX and the AUC ratios of MEGX to lidocaine in healthy and inflamed rats were observed. This indicates that acute inflammation caused by the cytokine had no effect on N-dealkylation of lidocaine to MEGX. Although other models of inflammation may influence CYP2C subfamily differently (Morgan, 1997), $IFN_{\alpha 2a}$ administered subcutaneous to rats in this study did not significantly alter lidocaine metabolism or MEGX formation. In support of this finding, our laboratory has reported acute inflammation caused by administration of $IFN_{\alpha 2a}$ did not alter presystemic clearance of nifedipine that is also metabolized in the rat by CYP2C subfamily (Eliot and Jamali, 1998; Smith, 1991). Similarly, the results of the *in vitro* study reflect those of intact rat, suggesting that acute inflammation caused by administration of the cytokine does not alter metabolism of lidocaine.

The present data indicates a selective effect of acute inflammation caused by administration of $IFN_{\alpha 2a}$ on presystemic metabolism of cardiovascular drug and responsiveness of cardiac ion channels. Interestingly, prolongation of the PR interval in the rat can be produced by both sodium and calcium channel blockade in the atrioventricular node (Penz *et al.*, 1992). Decreased prolongation of the PR interval was observed with inflamed rats administered verapamil indicating reduced sensitivity of cardiac calcium channels in the atrioventricular node. This perhaps, is due to alterations in calcium channel configuration, density or chemical messenger activity (Opie, 1998). Unlike verapamil, prolongation of the PR interval after lidocaine administration was not significantly different for inflamed compared to healthy rats suggesting that acute

inflammation caused by $IFN_{\alpha 2a}$ treatment selectively altered cardiac ion channel activity in the atrioventricular node.

The selective effect of acute inflammation on PR interval responsiveness to cardiovascular drug was accompanied by similar heart rate reductions in healthy and inflamed rats administered lidocaine and verapamil. Interestingly, multiple regulatory mechanisms control heart rate such as sympathetic and parasympathetic nervous systems and various local chemical messengers (Opie, 1998). Thus, an inflammation-induced alteration in heart rate response was not observed due, perhaps, to these complex physiological mechanisms adjusting heart rate responses to verapamil and lidocaine in healthy and inflamed rats.

Similar to the PR interval and heart rate, responsiveness of QT intervals to lidocaine was not influenced by $IFN_{\alpha 2a}$-induced inflammation. Verapamil had no effect on the QT interval. This was anticipated since verapamil blocks L-type calcium channels, slows conduction and prolongs refractoriness in the atrioventricular node (McEvoy, 1999). Previously we had reported that $IFN_{\alpha 2a}$-induced acute inflammation reduced responsiveness of cardiac potassium channels to β-adrenergic/potassium channel blocker, sotalol (Kulmatycki and Jamali, 1997). Pharmacokinetics of sotalol was not altered by inflammation thus decreased prolongation of the QT interval in the inflamed rat observed after administration of sotalol indicated a reduced cardiac potassium channel response. The lack of effect of $IFN_{\alpha 2a}$ on sodium channels suggests a selective effect of the cytokine-induced inflammation on ventricular ion channel activity since similar to sotalol, pharmacokinetics of lidocaine were not altered by the pro-inflammatory cytokine.

Therefore, similar pharmacokinetics and pharmacodynamics of lidocaine in healthy and $IFN_{\alpha 2a}$ inoculated rats indicates a selective effect of the cytokine-induced inflammation on CYP isozymes and cardiovascular receptor.

4.3 Conclusions

As expected inflammation caused a significant decrease in verapamil clearance but reduced the effect of drug on PR interval. A selective effect of inflammation on CYP isozyme and cardiac ion channel function was observed since lidocaine pharmacokinetics and pharmacodynamics was not altered by the cytokine-induced inflammation. Therefore, effect of inflammation on pharmacokinetics-pharmacodynamics of cardiovascular drug can not be generalized since the influence of inflammatory disease on drug disposition and activity is not universal.

Table 4-1. Hematological parameters after administration of 2 doses of $IFN_{\alpha 2a}$ 5.0×10^4 units subcutaneous for *in vivo* and *in vitro* experiments. Values are expressed as mean ± standard deviation, *denotes significantly different from control ($p<0.05$).

Parameter (%)	Control	$IFN_{\alpha 2a}$
Rats used for *in vivo* experiment		
Lymphocytes	78 ± 7	77 ± 2
Neutrophils	18 ± 5	13 ± 2
Segmented Neutrophils	4 ± 3	10 ± 2*
Rats used for *in vitro* experiment		
Lymphocytes	80 ± 6	82 ± 6
Neutrophils	15 ± 4	5 ± 2*
Segmented Neutrophils	5 ± 1	13 ± 5*

Table 4-2: Pharmacokinetic indices following oral administration of lidocaine (90 mg/kg) to control and inflamed rats. Values are expressed as mean ± standard deviation, no significant differences were found between control and $IFN_{\alpha 2a}$ inoculated rats ($p>0.05$).

	Placebo	$IFN_{\alpha 2a}$ Treatment
C_{max} (μg/ml)[a]	2.44 ± 1.43	1.87 ± 1.04
T_{max} (h)[b]	0.36 ± 0.07	0.46 ± 0.19
$t_{1/2}$ (h)[c]	1.15 ± 0.57	0.86 ± 0.31
$AUC_{0\text{-}3h}$ (μg*h/ml)[d]	2.57 ± 1.14	1.87 ± 0.54
$AUC_{0\text{-}\infty}$ (μg*h/ml)[e]	3.00 ± 1.30	2.12 ± 0.48
CL/F (L/h)[f]	11.67 ± 5.64	16.63 ± 3.96
Vd/F (L)[g]	17.79 ± 10.28	21.71 ± 11.65
MEGX,T_{max} (h)	0.49 ± 0.15	0.57 ± 0.26
MEGX,C_{max} (μg/ml)	4.50 ± 1.77	4.57 ± 1.69
MEGX,$AUC_{0\text{-}3h}$ (μg*h/ml)	8.14 ± 2.09	7.89 ± 2.73
AUC Ratio[h]	3.50 ± 1.16	4.18 ± 0.79

[a] Maximum observable plasma concentration
[b] Time at maximum observable plasma concentration
[c] Terminal elimination half-life estimated from at least 3 data points of each rat
[d] Truncated area under the plasma concentration-time curve from 0 to 3 h
[e] Area under the plasma concentration-time curve extrapolated to infinity
[f] Oral clearance
[g] Oral volume of distribution
[h] AUC Ratio of MEGX/Lidocaine

Table 4-3: Truncated area under the effect curves from 0-3 h following administration of lidocaine (90 mg/kg) to control and $IFN_{\alpha 2a}$ inoculated rats. Values are expressed as mean ± standard deviation, no significant differences were noted between healthy and inflamed rats ($p>0.05$).

	PR Interval (msec × h)		QT Interval (msec × h)		Heart Rate (msec × h)	
	Control	$IFN_{\alpha 2a}$	Control	$IFN_{\alpha 2a}$	Control	$IFN_{\alpha 2a}$
Lidocaine	250 ± 13	261 ± 41	376 ± 41	378 ± 36	2063 ± 156	1941 ± 211

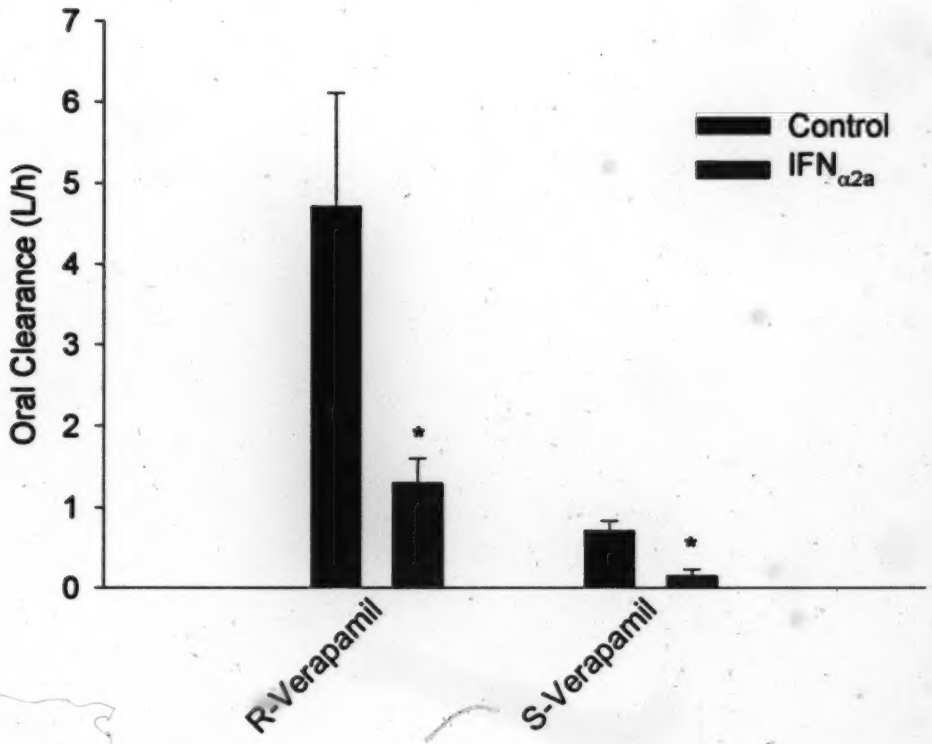

Figure 4-1: Effect of inflammation on verapamil oral clearance following administration of verapamil (20 mg/kg) to healthy and inflamed rats. Error bars represent standard deviation of the mean, *denotes significantly different between control and inflamed rats ($P<0.05$).

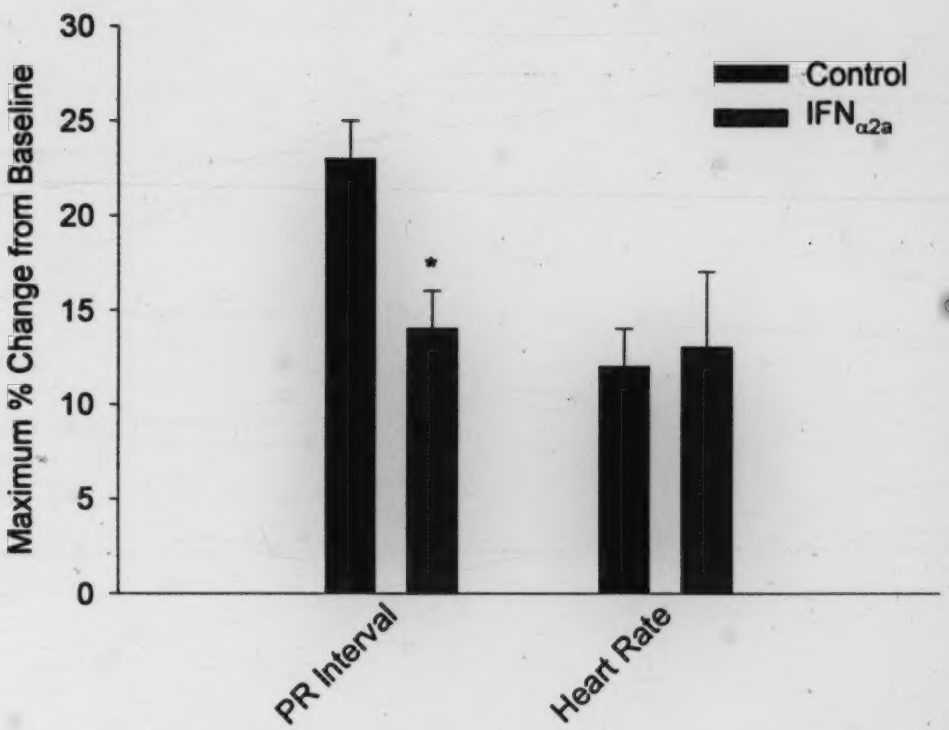

Figure 4-2: Effect of inflammation on PR interval prolongation and heart rate reduction following administration of verapamil (20 mg/kg) to healthy and inflamed rats. Error bars represent standard deviation of the mean, *denotes significantly different between control and inflamed rats ($p<0.05$).

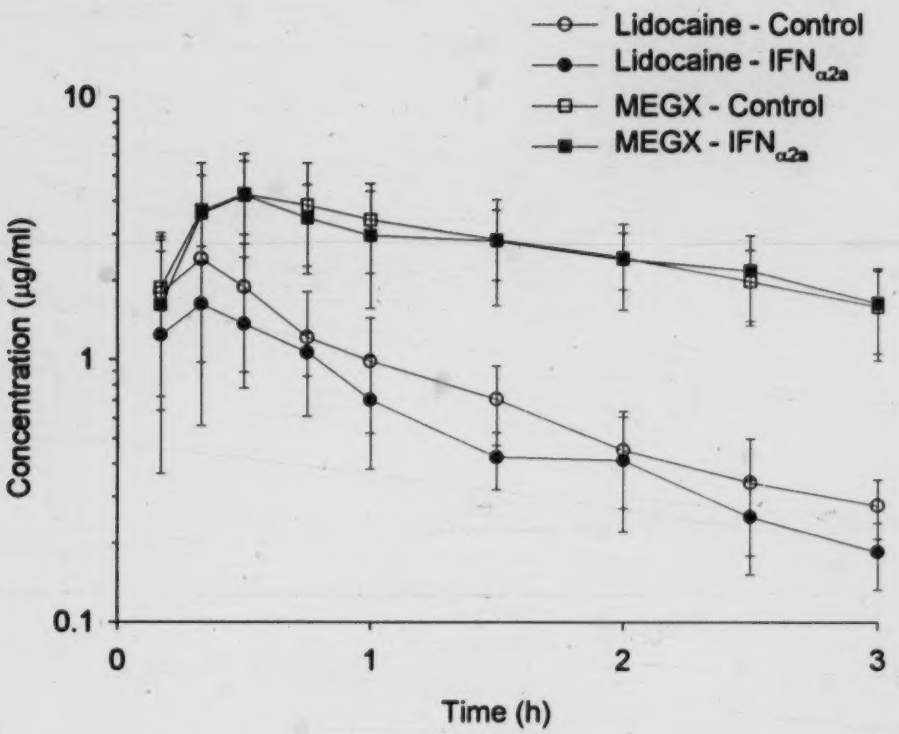

Figure 4-3: **Plasma lidocaine and MEGX concentration-time plots for control and inflamed rats following oral administration of 90 mg/kg lidocaine. Plasma concentrations are expressed as mean ± standard deviation. No significant differences between control and inflamed rats are observed ($p>0.05$).**

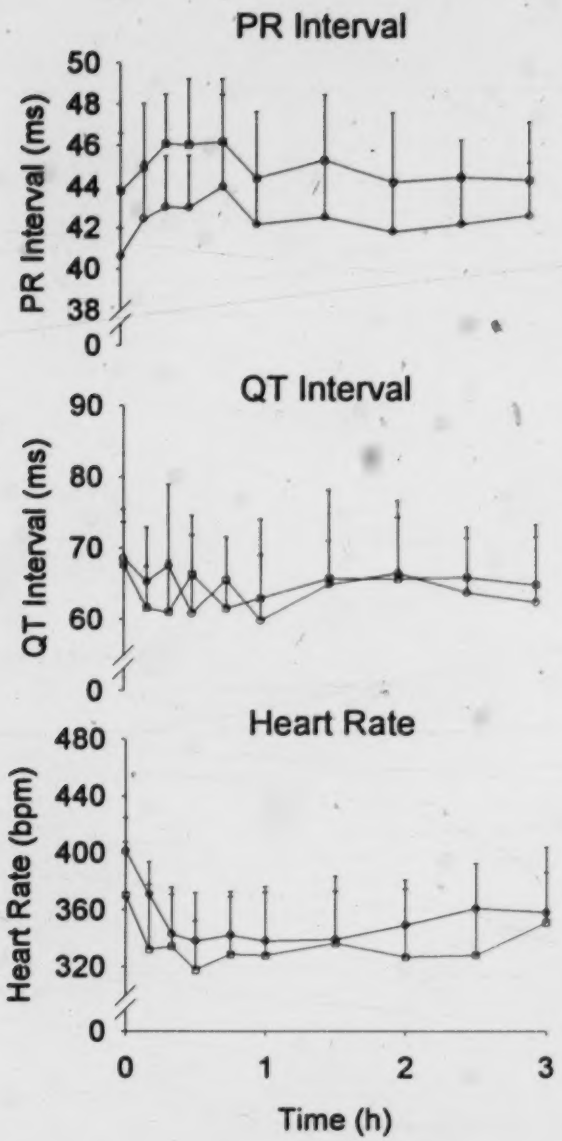

Figure 4-4: Time courses of lidocaine effect following administration of single oral doses of lidocaine (90 mg/kg) to healthy and inflamed rats. Control (O) and inflamed (□), error bars represent standard deviation of the mean, no significant differences were noted ($p>0.05$).

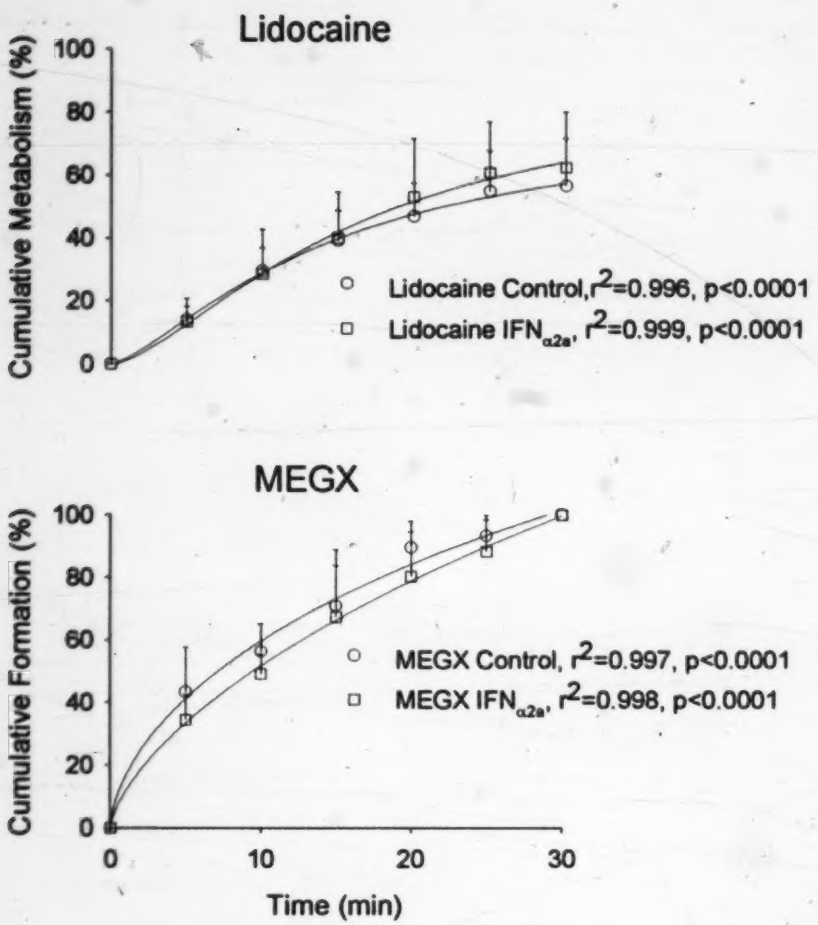

Figure 4-5: Time courses of microsome metabolism of lidocaine in healthy and $IFN_{\alpha 2a}$ inoculated rats (n=6/group). Cumulative percent metabolism of lidocaine and percent of total MEGX formed during incubation at substrate concentration of 2.5 μg/ml. Error bars represent standard deviation of the mean, lines through the data points represent the best fit lines.

Chapter 5

GENERAL DISCUSSIONS AND CONCLUSIONS

5.1 History

Greater concentrations of drug in inflammatory disease were discovered serendipitously during a study of propranolol absorption in patients with celiac disease (Schneider *et al.*, 1976). Patients with Crohn's disease and rheumatoid arthritis have also been reported to have higher concentrations of propranolol than healthy individuals (Schneider *et al.*, 1979). These greater concentrations of drug in rheumatoid arthritis and Crohn's patients seemed to be related to the ESR a nonspecific marker of inflammation. Shortly after these observations altered disposition of propranolol and other cardiovascular drugs in individuals with various types of inflammatory disorders were reported (Kendall *et al.*, 1979; Schneider *et al.*, 1981). In addition to human studies inflammation-induced changes in pharmacokinetics of cardiovascular drugs have also been observed in laboratory animals (Belpaire *et al.*, 1989; Piquette-Miller and Jamali, 1993).

Mechanisms proposed for these greater drug concentrations in laboratory animals and patients with inflammatory disease were greater binding of drug to plasma proteins and reduced intrinsic hepatic clearance. Piafsky *et al.*, in 1978 reported that the acute-phase protein AAG was elevated in individuals with Crohn's disease and rheumatoid arthritis. Interestingly, greater concentration of AAG in Crohn's disease and rheumatoid arthritis was negatively correlated with free fraction of propranolol (Piafsky *et al.*, 1978). With this in mind, elevated drug concentrations in inflammation were thought to be due to increased binding to plasma proteins (Walker *et al.*, 1986). However, altered disposition of cardiovascular drug in laboratory animals with inflammation has also been explained by a reduction in drug metabolism. Belpaire *et al.*, in 1989 reported that

presystemic clearance of metoprolol was less in rats with turpentine-induced arthritis. Metoprolol is negligibly bound to plasma proteins and undergoes extensive first-pass metabolism the reduced oral clearance observed in the arthritic rat was explained by a reduction in intrinsic hepatic clearance (Belpaire *et al.*, 1989). A possible explanation for the reduced metabolism of drug is increased expression of pro-inflammatory cytokines causing an elevation of nitric oxide which, in turn, oxidizes heme groups on CYP isozymes inhibiting enzyme activity (Morgan, 1997). Interestingly, a positive correlation between AUC of propranolol enantiomers and severity of adjuvant arthritis has been reported (Piquette-Miller and Jamali, 1995) thus severity of inflammation contributes to the extent of the reduced oral clearance of drug. Therefore, greater concentrations of drug in laboratory animals and patients with inflammatory disease are attributed to increased binding of drug to plasma proteins and reduced hepatic clearance.

Despite concern expressed in 1982 over well-being of patients with inflammatory disease who have greater concentrations of these cardiovascular drugs few studies to date have examined the effect of inflammation on drug responsiveness (Schneider and Bishop, 1982). Three studies reported a reduced β-adrenergic response to propranolol in adjuvant rats treated with isoproterenol. It was thought that the reduced responsive to propranolol was due to an increased binding of propranolol to AAG preventing drug from interacting with β-adrenergic receptors (Belpaire *et al.*, 1986; Barber *et al.*, 1983; Walker *et al.*, 1986). Recently the effect of inflammation on pharmacokinetics and pharmacodynamics of verapamil and propranolol were investigated (Mayo *et al.*, 1996; Guirguis and Jamali, 1996). Greater concentrations of verapamil enantiomers were observed in laboratory rats with $IFN_{\alpha 2a}$-induced inflammation compared to controls (Mayo *et al.*, 1996).

Interestingly, despite elevated concentrations of verapamil stereoisomers in rats with inflammation, responsiveness of cardiac calcium channels to verapamil was decreased in the inflamed rat compared to controls. Greater concentrations of propranolol are observed in laboratory rats with inflammation compared to controls (Piquette-Miller and Jamali, 1993). Despite higher drug concentrations in rats with inflammation, a reduced responsiveness of β-adrenergic receptors to propranolol was observed (Guirguis and Jamali, 1996). Interestingly, treatment with an NSAID [i.e., ketoprofen] reduced inflammation and normalized propranolol pharmacokinetics, however, the PR interval was still decreased after propranolol administration, comparing healthy to arthritis rats, indicating reduced responsiveness of β-adrenergic receptors (Guirguis and Jamali, 1996).

It was not known whether this reduced responsiveness of cardiac calcium channels and β-adrenergic receptors is due to increased protein binding or reduced receptor responsiveness since both verapamil and propranolol are highly protein bound. In addition, the diminished activity of verapamil and propranolol observed in the presence of inflammation is opposite to what is commonly seen and understood since higher concentration of drug is considered to cause either greater effect or toxicity. This suggests that concentration-effect relationships in inflammatory disease can not be fitted to typical pharmacokinetic-pharmacodynamic curves. Therefore, additional studies that examine the effect of inflammation on drug activity and disposition are crucial in order to understand the mechanisms responsible for increased concentration and altered response to drug in individuals and laboratory animals with inflammatory disease.

5.2 Pharmacokinetics-pharmacodynamics of sotalol

Adjuvant arthritis and $IFN_{\alpha 2a}$-induced inflammation altered ECG responses to sotalol after administration of both racemate and stereoisomers. The altered response to drug may possibly be explained by changes in the unbound or total drug concentrations. However, no pharmacokinetic differences are noted between control and inflamed rats after administration of racemate and enantiomer. Inflammation has been shown to result in increased serum AAG and decreased intrinsic hepatic clearance (Piquette-Miller and Jamali, 1995). Since sotalol binds to plasma proteins only to a limited degree (<2%) (Belpaire *et al.*, 1982) and is predominantly cleared through the renal pathway, the lack of effect of inflammation is not unexpected. Therefore, the observed changes in pharmacodynamics may be attributed to altered receptor sensitivity. Interestingly, this suggests the reduced responsiveness of β-adrenergic and cardiac calcium channels to propranolol and verapamil respectively is due to alterations in receptor function rather than protein binding.

Adjuvant arthritis and inflammation caused by inoculation with $IFN_{\alpha 2a}$ resulted in reduced β-adrenergic response to sotalol. This decreased β-adrenergic responsiveness may be due to the presence of pro-inflammatory cytokines causing an alteration in β-adrenergic receptor structure, density or chemical messenger activity. By taking advantage of stereochemistry and administering S-sotalol, pure potassium channel blocker, the effect of inflammation on cardiac potassium channels was confirmed. Reduced cardiac potassium channel sensitivity to S-enantiomer was observed in $IFN_{\alpha 2a}$ treated rat. Pro-inflammatory mediators released by the inflammatory response may

change function or number of cardiac potassium channels. Therefore, the altered response to drug in the inflamed rat may be attributed to the influence of pro-inflammatory cytokines on the action of sotalol on both the β-adrenergic and potassium channel receptors.

A decreased β-adrenergic and cardiac potassium channel response to sotalol was observed in both acute, $IFN_{\alpha 2a}$-induced, and chronic, *Mycobacterium butyricum*-induced, models of inflammation. Inflammation caused by injection of *Mycobacterium butyricum* resulted in greater inflammatory responses and tissue damage than observed after cytokine inoculation. Interestingly, sotalol pharmacodynamics were affected to a similar extent by both chronic and acute inflammatory disease despite inherent differences between these two models of inflammation.

5.2.1. Clinical implications of inflammation on sotalol

Administration of β-adrenergic antagonists have been found to be less efficacious in treating hypertension in older than younger individuals (Messerli *et al.*, 1998). Decreased prolongation of the PR interval indicating reduced sensitivity of cardiac calcium channels to verapamil has been reported in older patients (Abernethy *et al.*, 1986). This, perhaps, is due to an increased expression of inflammatory cytokines affecting cardiac calcium channel activity since increased concentrations certain pro-inflammatory cytokines (i.e., TNFα, IL-1) are observed in the elderly (Bruunsgaard *et al.*, 1999; Liao *et al.*, 1993). In addition, altered adrenergic sensitivity to β-blockers has

also been shown to occur with advancing age as seen with propranolol (Brodde *et al.*, 1995; Tenero *et al.*, 1990). The Medical Research Council of Britain reported that elderly patients treated for hypertension with atenolol, a cardioselective β_1-adrenergic antagonist, experienced no benefits (MRC Working Party, 1992). Clinically, therefore, reduced β-adrenergic receptor response may occur in various circumstances not only with individuals having inflammatory conditions such as arthritis, Crohn's disease, infection, but also in the elderly.

The reduced QT response to sotalol observed in the inflamed rat may be due to an inflammation-induced alteration of cardiac potassium channel function since no changes in pharmacokinetics was observed. The pro-inflammatory mediators released by the inflamed tissue may alter the function or number of active channels. This reduced sensitivity may affect not only effectiveness, but also adverse effects such as *torsades des pointes*. *Torsades des pointes* are excessive prolongation of QT intervals that cause a polymorphic ventricular tachycardia, which may lead to ventricular fibrillation (McEvoy, 1999). This effect is suggested to be dose related; i.e., approximately 0.5% and 6% after low and higher doses respectively (McEvoy, 1999). Therefore, perhaps the incidence of this side effect is less in individuals with inflammatory disease.

In support of our findings in the rat, the pro-inflammatory state is reported to be a major factor in the prognosis of individuals with cardiovascular diseases who received standard drug treatment. For example, inflammatory status has been shown to be a main determinant in the outcome of patients with unstable angina (Verheggen *et al.*, 1999). Patients with unstable angina who had significantly greater concentrations of inflammatory mediators CRP, fibrinogen, and ESR were refractory to standard therapy.

In addition, serum CRP concentration and mortality after acute myocardial infarction was investigated (Pietila *et al.*, 1996). Patients with significantly higher CRP concentrations, a marker of inflammation, treated with thrombolytic drugs had an increased mortality up to 6 months post myocardial infarction. Thus, despite drug treatment a higher mortality rate was observed in these patients. These two studies indicate that inflammatory disease influences response to conventional medical therapy. Therefore, inflammatory status of the patient due to disease or aging may have a significant impact on response to drug, hence, patient outcomes.

5.3 Pharmacokinetics-pharmacodynamics lidocaine

Inflammation caused by administration of $IFN_{\alpha 2a}$ had no effect on lidocaine pharmacokinetics. This is surprising since lidocaine undergoes extensive first-pass metabolism is highly protein bound and inflammatory disease is considered to influence drugs which have these characteristics (Belpaire *et al.*, 1989; Piquette-Miller and Jamali, 1995). Although administration of the cytokine did not alter the disposition of lidocaine, however, pharmacokinetics of verapamil were changed. The lack of effect of $IFN_{\alpha 2a}$-induced inflammation on pharmacokinetics of lidocaine may perhaps be explained by isozyme specificity since in the rat lidocaine is mainly metabolized by CYP2C subfamily [i.e., CYP2C11]. In support of this finding, similar plasma concentrations of nifedipine were observed in healthy and $IFN_{\alpha 2a}$ treated rats (Eliot and Jamali, 1998). Nifedipine like lidocaine is metabolized by CYP2C subfamily in rats (Smith, 1991).

ECG responses to lidocaine in healthy and inflamed rats were not different. This indicates a selective effect of inflammation on cardiovascular receptor since pharmacokinetics of lidocaine were not affect by the inflammation. Therefore, cardiac sodium channel responsiveness to drug is not altered by inflammation caused by administration of $IFN_{\alpha 2a}$.

5.3.1. Clinical implications of inflammation on lidocaine

The lack of effect of inflammation caused by $IFN_{\alpha 2a}$ administration on disposition and responsiveness of cardiac sodium channels to lidocaine indicates a selective effect of acute inflammation on cardiovascular receptors and isozymes. Interestingly, the influences of inflammatory disease observed with propranolol and verapamil pharmacokinetics and pharmacodynamics do not occur with lidocaine. Therefore, generalizing the effect of inflammation on drug disposition and activities may subject patients to experiencing inappropriate pharmacotherapy.

5.4 Conclusions

1. Adjuvant arthritis and $IFN_{\alpha 2a}$-induced inflammation did not alter pharmacokinetics of sotalol enantiomers after administration of racemate and stereoisomers. Hence, both acute and chronic models of inflammation had a similar effect on disposition of sotalol. This was expected since sotalol is negligibly bound to plasma proteins and eliminated via the renal route.

2. Pharmacokinetics of sotalol enantiomers were not altered by either acute or chronic inflammatory disease. This suggests the altered response to drug observed in the rat with inflammation is due to pharmacodynamic rather than pharmacokinetic changes.

3. Responsiveness of β-adrenergic receptors to sotalol is decreased in rats with inflammatory disease observed by a reduced prolongation of the PR interval in rats with adjuvant arthritis and $IFN_{\alpha 2a}$-induced inflammation compared to controls. The reduced β-adrenergic function in the inflamed rat is in agreement with *in vitro* observations of less activity of β-adrenergic receptors in the presence of pro-inflammatory cytokines.

4. Cardiac potassium channel responsiveness to sotalol was reduced in adjuvant arthritic rats and $IFN_{\alpha 2a}$ treated rats compared to controls. The reduced cardiac potassium channel activity in the cytokine and adjuvant treated rat is similar to *in vitro* observation of reduced cardiac potassium channel function in the presence of $IFN_{\alpha 2a}$.

5. Reduced cardiac potassium channel and β-adrenergic responsiveness to sotalol observed in rats with acute, $IFN_{\alpha 2a}$-induced, and chronic, *Mycobacterium butyricum*-induced, inflammatory disease was similar.

6. Pharmacokinetic observation may not necessarily reflect pharmacodynamic outcome since a decreased response to sotalol was observed in rats with inflammation despite unaltered pharmacokinetics. Thus, indiscriminate use of pharmacokinetics to predict drug response may result in inappropriate pharmacotherapy.

7. Despite elevated concentrations of verapamil after administration of $IFN_{\alpha 2a}$, disposition of lidocaine and formation of MEGX was similar in healthy and cytokine inoculated rats. This is due perhaps to isozyme specificity since CYP2C subfamily [i.e. CYP2C11] mainly catalyzes breakdown of lidocaine to MEGX.

8. Cardiac calcium channel response to verapamil was reduced in the cytokine treated rat. However, responsiveness of cardiac sodium channels to lidocaine is not altered by administration of the cytokine. This suggests a selective effect of $IFN_{\alpha 2a}$-induced inflammation on cardiac ion channel function.

REFERENCE LIST

Abernethy DR, Schwartz JB, Todd EL, Luchi R and Snow E (1986) Verapamil pharmacodynamics and disposition in young and elderly hypertensive patients altered electrocardiographic and hypotensive responses. *Ann Intern Med* **105**:329-336.

Abernethy DR and Schwartz JB (1988) Verapamil pharmacodynamics and disposition in obese hypertensive patients. *J Cardiovasc Pharmacol* **11**:209-215.

Abernethy DR, Wainer IW, Longstreth JA and Andrawis NS (1993) Stereoselective verapamil disposition and dynamics in aging during racemic verapamil administration. *J Pharmacol Exp Ther* **266**:904-911.

Advani SV and Singh BN (1995) Pharmacodynamic, pharmacokinetic and antiarrhythmic properties of *d*-sotalol, the dextro-isomer of sotalol. *Drugs* **49**:664-679.

Aggarwal S, Tsuruo T and Gupta S (1997) Altered expression and function of P-glycoprotein (170 kDa), encoded by the *MDR 1* gene, in T cell subsets from aging humans. *J Clin Immunol* **17**:448-454.

Alexander WR, Schlant RC and Fuster V eds (1998) *Hurst's the heart arteries and veins*. McGraw-Hill, New York.

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I and Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol* **39**:361-398.

Anderson JL and Prystowsky EN (1999) Sotalol: an important new antiarrhythmic. *Am Heart J* **137:**388-409.

Anker SD, Ponikowski PP, Clark AL, Leyva F, Rauchhaus M, Kemp M, Teixeira MM, Hellewell PG, Hooper J, Poole-Wilson PA and Coats AJS (1999) Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure. *Eur Heart J* **20:**683-693.

Argiles JM, Lopez-Soriano J, Busquets S and Lopez-Soriano FJ (1997) Journey from cachexia to obesity by TNF. *FASEB J* **11:**743-751.

Baert FJ, D'haens GR, Peeters M, Hiele MI, Schaible TF, Shealy D, Geboes K and Rutgeerts PJ (1999) Tumor necrosis factor α antibody (Infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* **116:**22-28.

Barber HE, Hawksworth GM and Walker KA (1983) The pharmacokinetics and pharmacodynamics of propranolol in rats with raised erythrocyte sedimentation rates. *Br J Pharmacol Pro Supp* **78:**60P.

Bargetzi MJ, Aoyama T, Gonzalez FJ, and Meyer UA (1989) Lidocaine metabolism in human liver microsomes by cytochrome P450IIIA4. *Clin Pharmacol Ther* **46:**521-527.

Bauman JL (1997) Class III antiarrhythmic agents: the next wave. *Pharmacotherapy* **17(2 Pt 2):**76S-83S.

Beckett AH, Boyes RN and Appleton PJ (1966) The metabolism and excretion of lignocaine in man. *J Pharm Pharmac* **18(Suppl):**76S-81S.

Belpaire FM, Bogaert MG and Rosseneu M (1982) Binding of β-adrenoceptor blocking drugs to human serum albumin, to α_1-acid glycoprotein and to human serum. *Eur J Clin Pharmacol* **22**:253-256.

Belpaire FM, Bogaert MG, Mugabo P and Rosseel MT (1986) Binding to serum α_1-acid glycoprotein and effect of β-adrenoceptor antagonists in rats with inflammation. *Br J Pharmac* **88**:697-705.

Belpaire FM, De Smet F, Chindavijak B, Fraeyman N and Bogaert MG (1989) Effect of turpentine-induced inflammation on the disposition kinetics of propranolol, metoprolol, and antipyrine in the rat. *Fundam Clin Pharmacol* **3**:79-88.

Berard JL, Velez RL, Freeman RB and Tsunoda SM (1999) A review of interleukin-2 receptor antagonists in solid organ transplantation. *Pharmacotherapy* **19**:1127-1137.

Biasucci LM, Liuzzo G, Fantuzzi G, Caligiuri G, Rebuzzi AG, Ginnetti F, Dinarello CA and Maseri A (1999) Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation* **99**:2079-2084.

Bondeson J (1997) The mechanism of action of disease-modifying antirheumatic drugs: a review with emphasis on macrophage signal transduction and the induction of proinflammatory cytokines. *Gen Pharmac* **29**:127-150.

Brodde OE, Zerkowski HR, Schranz D, Broede-Sitz A, Michel-Reher M, Schafer-Beisenbusch E, Piotrowski JA and Oelert H (1995) Age-dependent changes in the β-adrenoceptor-G-protein(s)-adenylyl cyclase system in human right atrium. *J Cardiovasc Pharmacol* **26**:20-26.

Bruunsgaard H, Andersen-Ranberg K, Juene B, Pedersen AN, Skinhoj P and Pedersen BK (1999) A high plasma concentration of TNF-α is associated with dementia in centenarians. *J Gerentol A Biol Sci Med Sci* **54A**:M357-M364.

Budavari S, ed (1996) *The Merck Index*. Merck Research Laboratories, New Jersey.

Burgot G, Serrand P and Burgot JL (1990) Thermodynamics of partitioning in the *n*-octanol/water system of some *β*-blockers. *Int J Pharm* **63**:73-76.

Burney RG, DiFazio CA, Peach MJ, Petrie KA and Silvester MJ (1974) Anti-arrhythmic effects of lidocaine metabolites. *Am Heart J* **88**:765-769.

Carr RA, Foster RT and Bhanji NH (1991) Stereospecific high-performance liquid chromatographic assay of sotalol in plasma. *Pharm Res* **8**:1195-1198.

Cassatella MA (1995) The production of cytokines by polymorphonuclear neutrophils. *Immunol Today* **16**:21-26.

Castleden CM, George CF and Short MD (1978) Contribution of individual differences in gastric emptying to variability in plasma propranolol concentrations. *Br J clin Pharmac* **5**:121-122.

Cawthorne MA, Palmer ED and Green J (1976) Adjuvant-induced arthritis and drug-metabolizing enzymes. *Biochem Pharmacol* **25**:2683-2688.

Chung MK, Gulick TS, Rotondo RE, Schreiner GF and Lange LG (1990) Mechanism of cytokine inhibition of β-adrenergic agonist stimulation of cyclic AMP in rat cardiac myocytes impairment of signal transduction. *Circ Res* **67**:753-763.

Cochran FR, Selph J and Sherman P (1996) Insights into the role of nitric oxide in inflammatory arthritis. *Med Res Rev* **16**:547-563.

Comini L, Bachetti T, Angoletti L, Gaia G, Curello S, Milanesi B, Volterrani M, Parrinello G, Ceconi C, Giordano A, Corti A and Ferrari R (1999) Induction of functional inducible nitric oxide synthase in monocytes of patients with congestive heart failure. *Eur Heart J* **20**:1503-1513.

Cooper BT and Lucas ML (1976) Propranolol absorption in Crohn's disease and coeliac disease. *Br Med J* **2**:1135.

Dahlgren C and Karlsson A (1999) Respiratory burst in human neutrophils. *J Immunol Methods* **232**:3-14.

Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A and Wadden T (1998) Tumor necrosis factor-α in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* **83**:2907-2910.

Davies NM and Jamali F (1997) Pharmacological protection of NSAID-induced intestinal permeability in the rat: effect of tempo and metronidazole as potential free radical scavengers. *Hum Exp Toxicol* **16**:345-349.

Davies CH, Ferrara N and Harding SE (1996) β-adrenoceptor function changes with age of subject in myocytes from non-failing human ventricle. *Cardiovasc Res* **31**:152-156.

Davies DH, Halablab MA, Clarke J, Cox FEG, and Young TWK (1999) The immune system, in *Infection and Immunity* pp 1-31, Taylor and Francis, Pennsylvania.

De Leve LD and Piafsky KM (1981) Clinical significance of plasma binding of basic drugs. *TIPS* **2**:283-284.

Dentino AN, Pieper CF, Rao KMK, Currie MS, Harris T, Blazer DG and Cohen HJ (1999) Association of interleukin-6 and other biological variables with depression in older people living in the community. *J Am Geriatr Soc* **47**:6-11.

Derendorf H and Meibohm B (1999) Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: concepts and perspectives. *Pharm Res* **16**:176-185.

Deswal A, Bozkurt B, Seta Y, Parilti-Eiswirth S, Hayes FA, Blosch C and Mann DL (1999) Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, Etanercept) in patients with advanced heart failure. *Circulation* **99**:3224-3226.

Detweiler DK (1981) The use of electrocardiograph in toxicology studies with rats, in *The rat electrocardiogram in pharmacology and toxicology* (Budden R, Detweiler DK and Zbinden G eds) pp 83-115, Pergamon Press, New York.

de Wildt SN, Kearns GL, Leeder JS and van den Anker JN (1999) Cytochrome P450 3A ontogeny and drug disposition. *Clin Pharmacokinet* **37**:485-505.

Doerge RF (1982) *Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry* p 845, Lippincott Company, Philadelphia.

Donnelly MT and Hawkey CJ (1997) Review article: COX-II inhibitors-a new generation of safer NSAIDs? *Aliment Pharmacol Ther* **11**:227-236.

Dorian P, Newman D, Sheahan R, Tang A, Green M and Mitchell J (1996) d-sotalol decreases defibrillation energy requirements in humans: a novel indication for drug therapy. *J Cardiovasc Electrophysiol* **7**:952-961.

Drach J, Gsur A, Hamilton G, Zhao S, Angerler J, Fiegl M, Zojer N, Raderer M, Haberl I, Andreeff M and Huber H (1996) Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4 and interferon-γ in normal human T lymphocytes. *Blood* **88**:1747-1754.

Dunn MJ and Hood VL (1977) Prostaglandins and the kidney. *Am J Physiol* **233**:F169-F184.

Eliot L and Jamali F (1998) Drug-Disease interactions: interferonα2a decreases nifedipine reflex tachycardia without altering pharmacokinetics. *Pharm Res* **1**:S470-471.

Esen F, Erdem T, Cakar N, Quintel M, Telci L, Akpir K and Van Ackern K (1997) Monoethylgylcinexylidide (MEGX) as an early predictor of liver dysfunction in severe sepsis. *Clin Int Care* **8**:260-266.

Feldmann M, Brennan FM and Maini RN (1996) Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* **14:**397-440.

Feldmann M, Bondeson J, Brennan FM, Foxwell BMJ and Maini RN (1999) The rationale for the current boom in anti-TNFα treatment. Is there an effective means to define therapeutic targets for drugs that provide all the benefits of anti-TNFα and minimize hazards? *Ann Rheum Dis* **58(Suppl I):**I27-I31.

Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti M-C, Cohen HJ, Penninx B, Pahor M, Wallace R and Havlik RJ (1999) Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc* **47:**639-647.

Fierro-Carrion GA and Ram CVS (1997) Nonsteroidal anti-inflammatory drugs (NSAIDs) and blood pressure. *Am J Cardiol* **80:**775-776.

Firestein GS (1999) Rheumatoid arthritis, in *Scientific American Medicine Online,* (Federman DD ed), Scientific American Inc., New York. Retrieved January 19, 2000 from the University of Alberta Library Databases (Academic Search Fulltext Elite) on the World Wide Web: http://www.library.ualberta.ca/library_html/database/elite.html

Flesch M, Kilter H, Cremers B, Laufs U, Sudkamp M, Ortmann M, Muller FU and Bohm M (1999) Effects of endotoxin on human myocardial contractility involvement of nitric oxide and peroxynitrite. *J Am Coll Cardiol* **33:**1062-1070.

Frishman W (1979) Clinical pharmacology of the new beta-adrenergic blocking drugs. Part 1. Pharmacodynamic and pharmacokinetic properties. *Am Heart J* **5:**663-670.

Fujita S, Tatsuno J, Kawai R, Kitagawa H, Suzuki T and Kitani K (1985) Age associated alteration of lidocaine metabolism is position selective. *Biochem Biophys Res Commun* **126**:117-122.

Gariepy L, Fenyves D and Villeneuve J-P (1992) Propranolol disposition in the rat: variation in hepatic extraction with unbound drug fraction. *J Pharm Sci* **81**:255-258.

Gibaldi M, Levy G and Weintraub H (1971) Drug distribution and pharmacologic effects. *Clin Pharmacol Ther* **12**:734-742.

Gibaldi M and Perrier D (1982) *Pharmacokinetics*. Marcel Dekker Inc., New York.

Gilbaldi M. (1991) *Biopharmaceutics and clinical pharmacokinetics.* Lea & Febiger, Philadelphia.

Gillespie WR (1991) Noncompartmental versus compartmental modelling in clinical pharmacokinetics. *Clin Pharmacokinet* **20**:253-262.

Goldberger AL (2000) Electrocardiography, in *Harrison's Online* (Braunwald E, Fauci A, Isselbacher KJ, Kasper DL, Hauser SL, Longo DL and Jamieson JL eds), McGraw-Hill, New York. Retrieved January 19, 2000 from the University of Alberta Databases (Academic Search Fulltext Elite) on the World Wide Web: http://www.library.ualberta.ca/library_html/databases/elite.html

Groeneveld PHP, Kwappenberg KMC, Langermans JAM, Nibbering PH and Curtis L (1997) Relation between pro- and anti-inflammatory cytokines and the production of nitric oxide (NO) in severe sepsis. *Cytokine* **9**:138-142.

Guillen I, Blanes M, Gomez-Lechon M-J and Castell JV (1995) Cytokine signaling during myocardial infarction: sequential appearance of IL-1β and IL-6. *Am J Physiol* **269:**R229-R235.

Guirguis M and Jamali F (1996) Propranolol pharmacodynamics in ketoprofen treated and untreated adjuvant arthritis. *Pharm Res* **13:**S444.

Guirguis M and Jamali F (1999) Is the difficulty in controlling blood pressure of patients with inflammatory disease NSAID related? *Pharm Res* **1:**S2048.

Gulick T, Chung MK, Pieper SJ, Lange LG and Schreiner GF (1989) Interleukin 1 and tumor necrosis factor inhibit cardiac myocyte β-adrenergic responsiveness. *Proc Natl Acad Sci USA* **86:**6753-6757.

Hackett LP, Wojnar-Horton RE, Dusci LJ, ILett KF and Roberts MJ (1990) Excretion of sotalol in breast milk. *Br J Clin Pharmac* **29:**277-278.

Hansen PS, Go MF, Varming K, Andersen LP, Genta RM, Graham DY and Nielsen H (1999) Proinflammatory activation of neutrophils and monocytes by *Helicobacter pylori* in patients with different clinical presentations. *Infect Immun* **67:**3171-3174.

Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr., Heimovitz H, Cohen HJ and Wallace R (1999) Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med* **106:**506-512.

Heales SJR, Bolanos JP, Stewart VC, Brookes PS, Land JM and Clark JB (1999) Nitric oxide, mitochondria and neurological disease. *Biochimica Biophysica Acta* **1410**:215-228.

Hirsch-Ernst KI, Ziemann C, Foth H, Kozian D, Schmitz-Salue C and Kahl GF (1998) Induction of mdr 1b mRNA and P-glycoprotein expression by tumor necrosis factor alpha in primary rat hepatocyte cultures. *J Cell Phy* **176**:506-515.

Hobbs AJ, Higgs A and Moncada S (1999) Inhibition of nitric oxide synthase as a potential therapeutic target. *Annu Rev Pharmacol Toxicol* **39**:191-220.

Hollunger G (1960) On the metabolism of lidocaine I. The properties of the enzyme system responsible for the oxidative metabolism of lidocaine II. The biotransformation of lidocaine. *Acta pharmacol et toxicol* **17**:356-373.

Ignarro LJ, Cirino G, Casini A and Napoli C (1999) Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* **34**:879-886.

Imaoka S, Enomoto K, Oda Y, Asada A, Fujimori M, Shimada T, Fujita S, Guengerich P and Funae Y (1990) Lidocaine metabolism by human cytochrome P-450s purified from hepatic microsomes: comparison of those with rat hepatic cytochrome P-450s. *J Pharmacol Exp Ther* **255**:1385-1391.

Irwin MW, Mak S, Mann DL, Qu R, Penninger JM, Yan A, Dawood F, Wen H, Shou Z and Liu P (1999) Tissue expression and immunolocalization of tumor necrosis factor-α in postinfarction dysfunctional myocardium. *Circulation* **99**:1492-1498.

Jack DB, Hawker JL, Rooney L, Beerahee M, Lobo J and Patel P (1988) Measurement of the distribution coefficients of several classes of drug using reversed-phase thin-layer chromatography. *J Chromatogr* **452**:257-264.

Johnson AG (1997) NSAIDs and increased blood pressure what is the clinical significance? *Drug Saf* **17**:277-289.

Johnson AG (1998) NSAIDs and blood pressure clinical importance for older patients. *Drugs & Aging* **12**:17-27.

Kaprielian R, Wickenden AD, Kassiri Z, Parker TG, Liu PP and PH Backx (1999) Relationship between K^+ channel down-regulation and $[Ca^{2+}]_i$ in rat ventricular myocytes following myocardial infarction. *J Physiol* **517**:229-245.

Kawai R, Fujita S and Suzuki T (1985) Simultaneous quantitation of lidocaine and its four metabolites by high-performance liquid chromatography: application to studies on in vitro and in vivo metabolism of lidocaine in rats. *J Pharm Sci* **74**:1219-1224.

Keenaghan JB and Boyes RN (1972) The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man. *J Pharmacol Exp Ther* **180**:454-463.

Kelley WN, Harris ED Jr., Ruddy S and Sledge CB eds (1997) *Textbook of rheumatology*, WB Saunders Company, Philadelphia.

Kendall MJ, Quarterman CP, Bishop H and Schneider RE (1979) Effects of inflammatory disease on plasma oxprenolol concentrations. *Br Med J* **2**:465-468.

Key BJ, Mucklow CA and Bishop H (1986) The absorption of propranolol from the jejunum in rats with adjuvant-induced arthritis. *Biopharm Drug Dispos* **7**:233-237.

Krich W, Spahn H, Ohnhaus EE, Kohler H, Heinz U and Mutschler E (1983) Influence of inflammatory disease on the clinical pharmacokinetics of atenolol and metoprolol. *Biopharm Drug Dispos* **4**:73-81.

Krown KA, Yasui K, Brooker MJ, Dubin AE, Nguyen C, Harris GL, McDonough PM, Glembotski CC, Palade PT and Sabbadini RA (1995) TNFα receptor expression in rat cardiac myocytes: TNFα inhibition of L-type Ca^{2+} current and Ca^{2+} transients. *FEBS Lett* **376**:24-30.

Kuby J (1997) Leukocyte migration and inflammation, in *Immunology* pp 357-378 WH Freeman and Company, New York.

Kulmatycki KM and Jamali F (1997) Pharmacodynamics of sotalol enantiomers are influenced by inflammation with no pharmacokinetic consequence. *Pharm Res* **14**:S511.

Labreque G and Belanger PM (1991) Biological rhythms in the absorption, distribution, metabolism and excretion of drugs. *Pharmac Ther* **52**:95-107.

Laethem ME, Belpaire FM, Wijnant P, Rosseel M-T and Bogaert MG (1994) Influence of endotoxin on the stereoselective pharmacokinetics of oxprenolol, propranolol, and verapamil in the rat. *Chirality* **6**:405-410.

Lagrand WK, Visser CA, Hermens WT, Niessen HWM, Verheugt FWA, Wolbink G-J and Hack E (1999) C-reactive protein as a cardiovascular risk factor more than an epiphenomenon? *Circulation* **100**:96-102.

Lefer AM and Lefer DJ (1994) Therapeutic role of nitric oxide donors in the treatment of cardiovascular disease. *Drugs Fut* **19**:665-672.

Le-Garrec LL, Delee E, Pascal JC and Jullien I (1987) Direct separation of d- and l-sotalol mandelate and hydrochloride salts by high performance liquid chromatography. *J Liquid Chromatogr* **10**:3015-3023.

Lenercept Multiple Sclerosis Study Group (1999) TNF neutralization in MS results of a randomized, placebo-controlled multicenter study. *Neurology* **53**:457-465.

Lennard MS, Tucker GT and Woods HF (1983) Time-dependent kinetics of lignocaine in the isolated perfused rat liver. *J Pharmacokinet Biopharm* **11**:165-182.

Lesko L and Williams RL (1994) Regulatory perspective: the role of pharmacokinetics and pharmacodynamics, in *Pharmacodynamics and drug development perspectives in clinical pharmacology* (Cutler NR, Sramek JJ and Narang PK eds) pp115-130, John Wiley & Sons, New York.

Levy G (1998) Predicting effective drug concentrations for individual patients determinants of pharmacodynamic variability. *Clin Pharmacokinet* **34**:323-333.

Liao Z, Tu JH, Small CB, Schnipper SM and Rosenstreich DL (1993) Increased urine interleukin-1 levels in aging. *Gerontology* **39**:19-27.

Lin A, Kenis G, Bignotti S, Tura GJB, De Jong R, Bosmans E, Pioli R, Altamura C, Scharpe S and Maes M (1998) The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. *Schizophr Res* **32:**9-15.

Little R, White MR and Hartshorn KL (1994) Interferon-α enhances neutrophil respiratory burst responses to stimulation with influenza A virus and FMLP. *JID* **170:**802-810.

Liu S and Schreur KD (1995) G protein-mediated suppression of L-type Ca^{2+} current by interleukin-1β in cultured rat ventricular myocytes. *Am J Physiol* **268:**C339-C349.

Liu SJ, Zhou W and Kennedy RH (1999) Suppression of β-adrenergic responsiveness of L-type Ca^{2+} current by IL-1β in rat ventricular myocytes. *Am J Physiol* **276:**H141-148.

Liuzzo G, Biasucci LM, Gallimore JR, Caligiuri G, Buffon A, Rebuzzi AG, Pepys MB and Maseri A (1999) Enhanced inflammatory response in patients with preinfarction unstable angina. *J Am Coll Cardiol* **34:**1696-1703.

Loubaris N, Cros G, Serrano JJ and Boucard M (1983) Circadian and circannual variation of the carregeenin inflammatory effect in rat. *Life Sci* **32:**1349-1354.

Lowry OH, Rosebrough NK, Farr AL and Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* **193:**265-275.

Lucas ML, Blair JA, Cooper BT and Cooke WT (1976) Relationship of the acid micro-climate in rat and human intestine to malabsorption. *Biochem Soc Trans* **4:**154-156.

MacFarlane LL, Orak DJ and Simpson WM (1995) NSAIDs, antihypertensive agents and loss of blood pressure control. *Am Fam Physician* **51**:849-856.

Maes M, Vandoolaeghe E, Ranjan R, Bosmans E, Bergmans R and Desnyder R (1995a) Increased serum interleukin-1-receptor-antagonist concentrations in major depression. *J Affect Disord* **36**:29-36.

Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R and Desnyder R (1995b) Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *J Affect Disord* **34**:301-309.

Maes M, Bosmans E, Kenis G, De Jong R, Smith RS and Meltzer HY (1997a) In vivo immunomodulatory effects of clozapine in schizophrenia. *Schizophr Res* **26**:221-225.

Maes M, Bosmans E, De Jong R, Kenis G, Vandoolaeghe E and Neels H (1997b) Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* **9**:853-858.

Malaguarnera M, Di Fazio I, Restuccia S, Pistone G, Ferlito L and Rampello L (1998) Interferon alpha-induced depression in chronic hepatitis C patients: comparison between different types of interferon alpha. *Neuropsychobiology* **37**:93-97.

Margaglione M, Cappucci G, Colaizzo D, Vecchione G, Grandone E, Di Minno G (2000) C-reactive protein in offspring is associated with the occurrence of myocardial infarction in first-degree relatives. *Arterioscler Thromb Vasc Biol* **20**:198-203.

Masubuchi Y, Umeda S, Chiba M, Fujita S and Suzuki T (1991) Selective 3-hydroxylation deficiency of lidocaine and its metabolite in Dark Agouti rats. *Biochem Pharmacol* **42**:693-695.

Masubuchi Y, Umeda S, Igarashi S, Fujita S, Narimatsu S, and Suzuki T (1993) Participation of the CYP2D subfamily in lidocaine 3-hydroxylation and formation of a reactive metabolite covalently bound to liver microsomal protein in rats. *Biochem pharmacol* **46**:1867-1869.

Mayo P, Kulmatycki K and Jamali F (1996) Pharmacokinetic interaction between interferonα2a and verapamil enantiomers without pharmacodynamic consequence. *Pharm Res* **13**:S434.

Mayo P and Jamali F (1999) Methoxyflurane anesthesia augments the chronotropic and dromotropic effects of verapamil. *J Pharm Pharmaceut Sci*, 2(1), 30-35. Retrieved January 7, 2000 on the World Wide Web: http://www.ualberta.ca/~csps.html

Mazoit JX, Orhant EE, Boico O, Kantelip J-P and Samii K (1993) Myocardial uptake of bupivacaine: I. Pharmacokinetics and pharmacodynamics of lidocaine and bupivacaine in the isolated perfused rabbit heart. *Anesth Analg* **77**:469-476.

McEvoy GK, ed. (1999) *American hospital formulary service drug information.* American Society of Health-System Pharmacists Inc., Bethesda.

Melzer E, Bardan E, Ronen I, Krepel Z, and Bar Meir S (1994) Alpha interferon has no effect on lidocaine metabolism in the rat. *Eur J Drug Metab Pharmacokinet* **2**:151-155.

Messerli FH, Grossman E and Goldbourt U (1998) Are β-blockers efficacious as first-line therapy for hypertension in the elderly? *JAMA* **279**:1903-1907.

Miossec P, Chomarat P and Dechanet J (1996) Bypassing the antigen to control rheumatoid arthritis. *Immunol Today* **17**:170-173.

Montegut W, Lowry SF and Moldawer LL (1995) Role of cytokines in septic shock and shock-related syndromes, in *Human cytokines: their role in disease and therapy* (Aggarwal BB and Puri RK eds) pp 381-390, Blackwell Science, Massachusetts.

Monteleone P, Fabrazzo M, Tortorella A and Maj M (1997) Plasma levels of interleukin-6 and tumor necrosis factor alpha in chronic schizophrenia: effects of clozapine treatment. *Psychiatry Res* **71**:11-17.

Morel PA and Oriss TB (1998) Crossregulation between Th1 and Th2 cells. *Crit Rev Immunol* **18**:275-303.

Morgan ET (1997) Regulation of cytochromes P450 during inflammation and infection. *Drug Metab Rev* **29**:1129-1188.

Morton DM and Chatfield DH (1970) The effects of adjuvant-induced arthritis on the liver metabolism of drugs in rats. *Biochem Pharmacol* **19**:473-481.

MRC Working Party (1992) Medical Research Council trial of treatment of hypertension in older adults: principal results. *BMJ* **304**:405-412.

Muller CM, Scierka A, Stiller RL, Kim Y-M, Cook DR, Lancaster JR, Buffington CW and Watkins WD (1996) Nitric oxide mediates hepatic cytochrome P450 dysfunction induced by endotoxin. *Anesthesiology* **84:**1435-1442.

Muller-Werdan U, Schumann H, Loppnow H, Fuchs R, Darmer D, Stadler J, Holtz J and Werdan K (1998) Endotoxin and tumor necrosis factor α exert a similar proinflammatory effect in neonatal rat cardiomyocytes, but have different cardiodepressant profiles. *J Mol Cell Cardiol* **30:**1027-1036.

Mulrow PJ (1999) Hypertension: a world wide epidemic, in *Hypertension primer the essentials of high blood pressure control basic science, population science, and clinical management* (Izzo JL Jr., and Black HR eds) pp 271-273, Lippincott Williams & Wilkins, Maryland.

Munoz-Fernandez MA and Fresno M (1998) The role of tumor necrosis factor, interleukin 6, interferon-γ and inducible nitric oxide synthase in the development and pathology of the nervous system. *Prog Neurobiol* **56:**307-340.

Murad F (1998) Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? *Rec Prog Horm Res* **53:**43-59.

Myles Glenn E, Bowman BJ, Rohloff NA and Seely RJ (1977) A major contributory cause of arthritis in adjuvant-inoculated rats: granulocytes. *Agents and Actions* **7:**265-282.

Nabauer M and Kaab S (1998) Potassium channel down-regulation in heart failure. *Cardiovasc Res* **37**:324-334.

Nakamoto T, Oda Y, Imaoka S, Funae Y and Fujimori M (1997) Effect of phenobarbital on the pharmacokinetics of lidocaine, monoethylgylcinexylidide and 3-hydroxylidocaine in the rat: correlation with P450 isoform levels. *Drug Metab Dispos* **25**:296-300.

Nashan B, Light S, Hardie IR, Lin A and Johnson JR for the daclizumab double therapy study group (1999) Reduction of acute renal allograft rejection by daclizumab. *Transplantation* **67**:110-115.

Naudin J, Mege JL, Azorin JM and Dassa D (1996) Elevated circulating levels of IL-6 in schizophrenia. *Schizophr Res* **20**:269-273.

Nelson SD, Garland WA, Breck GD and Trager WF (1977) Quantification of lidocaine and several metabolites utilizing chemical-ionization mass spectrometry and stable isotope labeling. *J Pharm Sci* **66**:1180-1189.

Nesher G and Ruchlemer R (1998) Alpha-interferon-induced arthritis: clinical presentation, treatment, and prevention. *Semin Arthritis Rheum* **27**:360-365.

Neumann F-J, Ott I, Gawaz M, Richardt G, Holzapfel H, Jochum M and Schomig A (1995) Cardiac release of cytokines and inflammatory responses in acute myocardial infarction. *Circulation* **92**:748-755.

Nikolaus S, Bauditz J, Gionchetti P, Witt C, Lochs H and Schreiber S (1998) Increased secretion of pro-inflammatory cytokines by circulating polymorphonuclear neutrophils and regulation by interleukin 10 during intestinal inflammation. *Gut* **42**:470-476.

Nishio M, Habuchi Y, Tanaka H, Morikawa J, Okanoue T, and Kashima K (1999) Tyrosine kinase-dependent modulation by interferon-α of the ATP-sensitive K^+ current in rabbit ventricular myocytes. *FEBS Lett* **445**:87-91.

Nolan PE (1997) Pharmacokinetics and pharmacodynamics of intravenous agents for ventricular arrhythmias. *Pharmacotherapy* **17(2 pt 2)**:65S-75S.

Nyberg G, Karlen B, Hedlund I, Grundin R and von Bahr C (1977) Extraction and metabolism of lidocaine in rat liver. *Acta pharmacol et toxicol* **40**:337-346.

Oda Y, Imaoka S, Nakahira Y, Asada A, Fujimori M, Fujita S and Funae Y (1989) Metabolism of lidocaine by purified rat liver microsomal cytochrome P-450 isozymes. *Biochem Pharmacol* **24**:4439-4444.

Opie LH (1998) Channels, Pumps, and exchangers, in *The heart physiology, from cell to circulation*. pp 71-114 Lippincott-Raven Publishers, Pennsylvania.

Parsons RL, Kaye CM, Raymond K, Trounce JR and Turner P (1976) Absorption of propranolol and practolol in coeliac disease. *Gut* **17**:139-143.

Patterson LH, Hall G, Nijar BS, Khatra PK and Cowan DA (1986) In-vitro metabolism of lignocaine to its *N*-oxide. *J Pharm Pharmacol* **38**:326.

Penz W, Pugsley M, Hsieh MZ, and Walker MJA (1992) A new ECG measure (RSh) for detecting possible sodium channel blockade in vivo in rats. *JPM* **27**:51-58.

Perner A and Rask-Madsen J (1999) Review article: the potential role of nitric oxide in chronic inflammatory bowel disorders. *Aliment Pharmacol Ther* **13**:135-144.

Petrovsky N, McNair P and Harrison LC (1998) Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. *Cytokine* **10**:307-312.

Piafsky KM, Borga O, Odar-Cederlof I, Johansson C and Sjoqvist F (1978) Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1-acid glycoprotein. *N Engl J Med* **299**:1435-1439.

Pieper JA and Johnson KE (1992) Lidocaine, in *Applied pharmacokinetics principles of therapeutic drug monitoring* (Evans WE, Schentag JJ and Jusko WJ eds) pp 21-1 to 21-37, Applied Therapeutics Inc., Washington.

Pietila KO, Harmoinen AP, Jokinitty J and Pasternack AI (1996) Serum C-reactive protein concentration in acute myocardial infarction and its relationship to mortality during 24 months of follow-up in patients under thrombolytic treatment. *Eur Heart J* **17**:1345-1349.

Piquette-Miller M and Jamali F (1992) Effect of adjuvant arthritis on the disposition of acebutolol enantiomers in rats. *Agents and Actions* **37**:290-296.

Piquette-Miller M and Jamali F (1993) Selective effect of adjuvant arthritis on the disposition of propranolol enantiomers in rats detected using a stereospecific HPLC assay. *Pharm Res* **10**:294-299.

Piquette-Miller M and Jamali F (1995) Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab Dispos* **23**:240-245.

Piquette-Miller M, Pak A, Kim H, Anari R and Shahzamani A (1998) Decreased expression and activity of P-glycoprotein in rat liver during acute inflammation. *Pharm Res* **15**:706-711.

Reglier H, Arce-Vicioso M, Fay M, Gougerot-Pocidalo MA and Chollet-Martin S (1998) Lack of IL-10 and IL-13 production by human polymorphonuclear neutrophils. *Cytokine* **10**:192-198.

Reichel C, Nacke A, Sudhop T, Wienkoop G, Luers C, Hahn C, Pohl C, Spengler U and Sauerbruch T (1997) The low-dose monoethylgylcinexylidide test: assessment of liver function with fewer side effects. *Hepatology* **25**:1323-1327.

Reynolds EF, ed (1996) *Martindale the Extra Pharmacopoeia*. Royal Pharmaceutical Society of Great Britain, Great Britain.

Rice G and Ebers G (1998) Interferons in the treatment of multiple sclerosis. Do they prevent the progression of the disease? *Arch Neurol* **55**:1578-1580.

Roden DM (1996) Antiarrhythmic drugs, in *Goodman and Gilman's The pharmacological basis of therapeutics* (Gilman A, Goodman S and Gilman A eds) pp 839-874, McGraw-Hill, New York.

Rohde LEP, Hennekens CH and Ridker PM (1999) Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am J Cardiol* **84:**1018-1022.

Rodighiero V (1989) Effects of cardiovascular disease on pharmacokinetics. *Cardiovasc Drugs Ther* **3:**711-730.

Rozanski GJ and Witt RC (1994) IL-1 inhibits β-adrenergic control of cardiac calcium current: role of L-arginine/nitric oxide pathway. *Am J Physiol* **267:**H1753-1758.

Ruoff GE (1998) The impact of nonsteroidal anti-inflammatory drugs on hypertension: alternative analgesics for patients at risk. *Clin Ther* **20:**376-387.

Schmucker DL and Vesell ES (1999) Are the elderly underrepresented in clinical drug trials? *J Clin Pharmacol* **39:**1103-1108.

Schneider RE, Babb J, Bishop H, Mitchard M, Hoare AM and Hawkins CF (1976) Plasma levels of propranolol in treated patients with coeliac disease and patients with Crohn's disease. *Br Med J* **2:**794-795.

Schneider RE, Bishop H and Hawkins CF (1979) Plasma propranolol concentrations and the erythrocyte sedimentation rate. *Br J Clin Pharmac* **8:**43-47.

Schneider RE, Bishop H, Kendall MJ and Quarterman CP (1981) Effect of inflammatory disease on plasma concentrations of three β-adrenoceptor blocking agents. *Int J Clin Pharmacol Ther Toxicol* **19**:158-162.

Schneider RE and Bishop H (1982) β-blocker plasma concentrations and inflammatory disease: clinical implications. *Clin Pharmacokinet* **7**:281-284.

Schultz R, Panas DL, Catena R, Moncada S, Olley PM and Lopaschuk G (1995) The role of nitric oxide in cardiac depression induced by interleukin-1β and tumor necrosis factor-α. *Br J Pharmacol* **114**:27-34.

Schwartz PJ (1998) Do animal models have clinical value? *Am J Cardiol* **81(6A)**:14D-20D.

Seta Y, Shan K, Bozkurt B, Oral H and Mann DL (1996) Basic mechanisms in heart failure: the cytokine hypothesis. *J Cardiac Failure* **2**:243-249.

Shand DG, Cotham RH and Wilkinson GR (1976) Perfusion-limited effects of plasma drug binding on hepatic drug extraction. *Life Sci* **19**:125-130.

Shibukawa A, and Wainer IW (1992) Simultaneous direct determination of the enantiomers of verapamil and norverapamil in plasma using a derivatized amylose high-performance liquid chromatographic chiral stationary phase. *J Chromatogr B Biomed Sci Appl* **574**:85-92.

Singh BN, Deedwania P, Nademanee K, Ward A and Sorkin EM (1987) Sotalol a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* **34:**311-349.

Smith DA (1991) Species differences in metabolism and pharmacokinetics: are we closer to an understanding? *Drug Metab Rev* **23:**355-373.

Stein U, Walther W and Shoemaker RH (1996) Modulation of mdr1 expression by cytokines in human colon carcinoma cells: an approach for reversal of multidrug resistance. *Br J Can* **74:**1384-1391.

Strong JM, Mayfield DE, Atkinson AJ Jr., Burris BC, Raymond F and Webster LT Jr. (1975) Pharmacological activity, metabolism, and pharmacokinetics of gylcinexylidide. *Clin Pharmacol Ther* **17:**184-194.

Supradist S, Notarianni LJ and Bennet PN (1984) Lignocaine kinetics in the rat. *J Pharm Pharmacol* **36:**240-243.

Suzuki T, Fujita S and Kawai R (1984) Precursor-metabolite interaction in the metabolism of lidocaine. *J Pharm Sci* **73:**136-138.

Tam YK, Tawfik SR, and Coutts RT (1987) High-performance liquid chromatography of lidocaine and nine of its metabolites in human plasma and urine. *J Chromatogr B Biomed Sci Appl* **423:**199-206.

Tenero DM, Bottorff MB, Burlew BS, Williams JB and Lalonde RL (1990) Altered β-adrenergic sensitivity and protein binding to l-propranolol in the elderly. *J Cardiovasc Pharmacol* **16**:702-707.

Tilg H and Peschel C (1996) Interferon-alpha and its effects on the cytokine cascade: a pro- and anti-inflammatory cytokine. *Leuk Lymphoma* **23**:55-60.

Torre-Amione G, Stetson SS and Farmer JA (1999) Clinical implications of tumor necrosis factor α antagonism in patients with congestive heart failure. *Ann Rheum Dis* **58(Suppl I)**:I103-I106.

Torreilles F, Salman-Tabcheh S, Guerin M-C and Torreilles (1999) Neurodegenerative disorders: the role of peroxynitrite. *Brain Res Rev* **30**:153-163.

Tselis AC and Lisak RP (1999) Multiple sclerosis therapeutic update. *Arch Neurol* **56**:277-280.

Tsujimoto G, Manger WM, and Hoffman BB (1984) Desensitization of β-adrenergic receptors by pheochromocytoma. *Endocrinology* **114**:1272-1278.

Ukawa H, Yamakuni H, Kato S and Takeuchi K (1998) Effects of cyclooxygenase-2 selective and nitric oxide-releasing nonsteroidal antiinflammatory drugs on mucosal ulcerogenic and healing responses of the stomach. *Dig Dis Sci* **43**:2003-2011.

van der Poll T and van Deventer SJH (1999) Cytokines and anticytokines in the pathogenesis of sepsis. *Infect Dis Clin North Am* **13**:413-426.

van Deventer SJH, Elson CO, and Fedorak RN for the Crohn's disease study group (1997) Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. *Gastroenterology* **113**:383-389.

van Hogezand RA and Verspaget HW (1998) The future role of anti-tumor necrosis factor-α products in the treatment of Crohn's disease. *Drugs* **56**:299-305.

Verheggen PWHM, de Maat MPM, Manger Cats V, Haverkate F, Zwinderman AH, Kluft C and Bruschke AVG (1999) Inflammatory status as a main determinant of outcome in patients with unstable angina, independent of coagulation activation and endothelial cell function. *Eur Heart J* **20**:567-574.

Vermeulen An M, Belpaire FM, De Smet F and Bogaert MG (1993) Influence of human recombinant interleukin-1β on the enantioselective disposition of propranolol in rats. *Biochem Pharmacol* **45**:1-6.

Visser M, Bouter LM, McQuillan GM, Wener MH and Harris TB (1999) Elevated C-reactive protein levels in overweight and obese adults. *JAMA* **282**:2131-2135

von Bahr C, Hedlund I, Karlen B, Backstrom D and Grasdalen H (1977) Evidence for two catalytically different binding sites of liver microsomal cytochrome P-450: importance for species and sex differences in oxidation pattern of lidocaine. *Acta pharmacol et toxicol* **41**:39-48.

Waldo AL, Camm AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt B, Pratt CM, Schwartz PJ and Enrico PV for the SWORD investigators (1996) Effect of *d*-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. *Lancet* **348**:7-12.

Walker KA, Barber HE and Hawksworth GM (1986) Mechanism responsible for altered propranolol disposition in adjuvant-induced arthritis in the rat. *Drug Metab Dispos* **14**:482-486.

Whelton A (1999) Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiologic foundations and clinical implications. *Am J Med* **106(5B)**:13S-24S.

Whitehouse MW, Orr KJ, Beck FWJ and Pearson CM (1974) Freund's adjuvants: relationship of arthritogenicity and adjuvanticity in rats to vehicle composition. *Immunology* **27**:311-330.

Whitehouse MW (1973) Abnormal drug metabolism in rats after an inflammatory insult. *Agents and Actions* **3**:312-316.

Whitehouse MW (1988) Adjuvant-induced polyarthritis in rats, in *CRC Handbook of animal models for the rheumatic diseases volume I* (Greenwald RA and Diamond HS eds) pp 3-16, CRC Press, Florida.

Wilkinson GR and Shand DG (1975) A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* **18**:377-390.

Xiao R-P, Tomhave ED, Wang D-J, Ji X, Boluyt MO, Cheng H, Lakatta EG and Koch WJ (1998) Age-associated reductions in cardiac β_1- and β_2-adrenergic responses without changes in inhibitory G proteins or receptor kinases. *J Clin Invest* **101**:1273-1282.

Yudkin JS, Stehouwer CDA, Emeis JJ and Coppack SW (1999) C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* **19**:972-978.

Yudoh K, Matsuno H, Nakazawa F, Yonezawa T and Kimura T (1999) Increased expression of multidrug resistance of P-glycoprotein on Th1 cells correlates with drug resistance in rheumatoid arthritis. *Arthritis Rheum* **42**:2014-2015.

Zhong J, Hwang T-C, Adams HR and Rubin LJ (1997) Reduced L-type calcium current in ventricular myocytes from endotoxemic guinea pigs. *Am J Physiol* **273**:H2312-H2324.